Biochemical Pharmacology, Vol. 24, pp. 2075–2081. Pergamon Press, 1975. Printed in Great Britain.

METABOLISM OF (^{14}C-1)-HALOTHANE *IN VIVO*—EFFECTS OF MULTIPLE HALOTHANE ANESTHESIA, PHENOBARBITAL AND CARBON TETRACHLORIDE PRETREATMENT*

EDWARD S. REYNOLDS† and MARY TREINEN MOSLEN

Departments of Pathology, Peter Bent Brigham Hospital and Harvard Medical School, Boston, Mass. 02115, U.S.A.

(*Received* 6 *November* 1974; *accepted* 21 *February* 1975)

Abstract—Neither single nor multiple halothane anesthesia at 2-day or 2-week intervals (0·85% for 5 hr) altered the metabolism by the rat *in vivo* of halothane ($^{14}CF_3CHBrCl$), as determined by the recovery of radioactivity in cold xylene and $^{14}CO_2$ from expired air and label in urine. Phenobarbital (PBT, 1 g/liter of drinking water for 30 days) pretreatment nearly halved the exhalation of halothane and doubled the excretion of metabolite in the urine, but did not increase or change the pattern of label incorporation in liver organelles or chemical constituents at 2 or 24 hr. Prior injury to the liver endoplasmic reticulum by carbon tetrachloride (CCl_4; 0·26 m-moles/kg) reduced both the amount of ^{14}C metabolite excreted in the urine and bound to the liver without changing the pattern of label incorporation. Relative proportions of total $^{14}CF_3CHBrCl$ dose recovered in the urine and liver fractions decreased slightly with each successive increase in dose over a 2000-fold range. Microsomal proteins but not lipids were selectively labeled. Among organelles, mitochondria incorporated the least label. The relationship of liver injury to the amount of halothane metabolized or bound to protein or lipid remains unclear. However, the results of this *in vivo* study are consistent with localizing the liver endoplasmic reticulum as the major site of halothane metabolism as well as the most likely site of injury by toxic metabolites of halothane.

Halothane, an effective inhalation anesthetic, is acknowledged to have hepatotoxic potential [1]. This halocarbon is metabolized *in vitro* by components of the mixed-function oxidase system (MFOS) of liver microsomes [2] and reactions of its metabolites with endoplasmic reticulum of the cell have been postulated to be the basis of its hepatotoxicity [3]. Phenobarbital (PBT), an inducer of certain components of the liver MFOS, not only enhances halothane metabolism *in vivo* and *in vitro* [4], but also increases its hepatotoxicity [5–8].

Although clinical studies have indicated that the frequency of liver injury after halothane administration is increased by prior exposure to this anesthetic agent [9–11], experiments with animals have not consistently provided support for this observation [3, 5, 12–18]. While Hughes and Lang [17] associated increased incidence of focal hepatic injury in guinea pigs with multiple halothane anesthesia at 2-week intervals, others employing different doses, time schedules and/or species have not been able to link re-anesthesia with enhancement of histologic or biochemical indicators of liver injury [5, 12, 18].

In prior studies we found that a single administration of anesthesia with halothane in PBT-pretreated animals produced histologic and biochemical injury and that, after repeated administration at 2-day intervals, its hepatotoxic effect was diminished [5]. In this study, metabolism and liver binding of halothane *in vivo* were examined over a 2000-fold dose range and found responsive to the effects of dose size, PBT pretreatment and prior injury to the liver endoplasmic reticulum by carbon tetrachloride (CCl_4). In contrast, its metabolism *in vivo* was not altered by prior halothane anesthesia.

EXPERIMENTAL

Animals. Male Charles River Rats (Wilmington, Mass.) of from 50 to 75 g and with free access to Purina Chow were given either sodium phenobarbital (1 g/liter) or an equimolar amount of NaCl in drinking water for 30 days. Daily consumption of PBT was approximately 10 mg/100 g rat. CCl_4-pretreated animals were fed 0·025 ml CCl_4/kg (0·26 m-mole/kg) by stomach tube 24 hr prior to halothane. Animals were housed in wire-bottom cages over processed clay animal litter.

Anesthesia. Rats fasted for 16 hr were anesthetized for 5 hr with 0·85% halothane as previously described [5].

Materials. Halothane-1-^{14}C (2 mCi/m-mole) Na_2-$^{14}CO_3$ (12 mCi/m-mole, Omnifluor and Aquafluor were purchased from New England Nuclear,

* Supported by United States Public Health Service Grants HL-06370 and AM-16183. A preliminary report of this investigation appeared in *Fedn Proc.* **33**, 219 (1974).

† Research Career Development Awardee (6-K-3-GM-7309) of the National Institutes of Health, U.S. Public Health Service.

Boston, Mass. Triton X-100 was obtained from Baker Chemical, Phillipsburg, N.J. All radioisotopes were stored in the dark at −16°.

Halothane-1-^{14}C according to the manufacturer had a single peak by gas chromatography (Carbowax 400 on Poracil C at 80°) which coincided with the single peak of radioactivity. For dose titration studies, 5 μCi halothane-1-^{14}C was diluted with appropriate amounts of nonradioactive anesthetic grade halothane to achieve dose levels of 0·1, 1 and 10 m-moles halothane/kg.

Metabolism of ^{14}*C-1-halothane* in vivo. Paired sets of PBT- and NaCl-pretreated rats were injected intraperitoneally with 5 μCi halothane-1-^{14}C in 0·025 ml hexane, or 20 μCi $Na_2{}^{14}CO_3$ in 0·10 ml of 0·01 M NaOH, placed immediately in all-glass metabolism cages (Delmar Roth, Maywood, Ill.) and given free access to food and water. Air flow was maintained at 0·5 liter/min. This amount of halothane did not produce narcosis or drowsiness. For studies of the expired label, metabolism cages were connected to a series of scrubbing towers, CO_2 was trapped in 2 N NaOH, moisture was removed by passage through a cold trap (−78°) and halothane-1-^{14}C was collected in cold xylene (after the method of Van Dyke *et al.* [19]). Total cold xylene-trappable radioactivity was considered "unmetabolized" halothane. Aliquots from the xylene traps were counted in Omnifluor (4 g/liter of toluene). CO_2 in NaOH traps was diffused to hydroxide of Hyamine in center wells of sealed Conway dishes after acidification of aliquots in outer wells with 12 N H_2SO_4 [20]. $^{14}CO_2$ trapped in Hyamine was then transferred to Omnifluor by successive methanol washes. Aliquots of urine collected were counted in toluene-based Omnifluor. Efficiencies of the trapping system were virtually 100 per cent when tested by placing samples of acidified $Na_2{}^{14}CO_3$, or $^{14}CF_3CHBrCl$ in the metabolism cages without animals and running the system as usual. For 24-hr urinary metabolite excretion studies, animals were placed in plastic metabolism cages open to room air. All samples for scintillation counting were corrected to 100 per cent efficiency by sample channels ratio techniques.

Hepatic labeling patterns. Livers of paired groups of PBT- or NaCl-pretreated rats given 5 μCi $^{14}CF_3CHBrCl$ intraperitoneally 2 or 24 hr previously were perfused with ice-cold 0·25 M sucrose, pulped in a hand press and then homogenized in 5 vol. of 0·25 M sucrose. Aliquots of homogenate were set aside and the remainder was fractionated into residue, mitochondria, microsomes and cell sap by differential centrifugation [21]. Aliquots of each were dissolved in Hyamine and counted in toluene-based Omnifluor. Proteins, nucleic acids, lipids and acid-soluble substances were isolated and analyzed as previously described [22]. All animals were fasted for 16 hr prior to sacrifice. Aliquots of soluble fractions were counted in Aquafluor, nucleic acids and proteins in toluene-based Omnifluor containing 25% Triton X-100, and lipids (previously taken to dryness under N_2) in Omnifluor.

Since preliminary studies showed no decrease in radioactivity upon repeated drying of aliquots of liver homogenate after successive additions of unlabeled halothane to remove any unmetabolized halocarbons present, the radioactivity present in cell fractions was considered to represent accurately the nonvolatile products of metabolism. For statistical analysis, Student's *t*-test was used as a measure of null hypothesis with $P < 0.05$.

RESULTS

Halothane excretion. After intraperitoneal administration of ^{14}C-1-halothane to NaCl-treated animals (controls), approximately half of the total radioactivity given was recovered in cold xylene within 8 hr and less than 1 per cent as $^{14}CO_2$ during the same period (Fig. 1). Excretion of label in urine was much slower; cumulative excretion in the urine gradually leveled off at one-quarter the total dose by the end of the first week (Fig. 2). PBT pretreatment nearly doubled the amount excreted in the urine and halved that recoverable as 'halothane' during these periods (Figs. 1 and 2).

Urinary excretion of halothane metabolites in both NaCl- and PBT-treated animals follows apparent first-order kinetics, with excretion of radioactivity diminishing by half every 36 hr during the 7-day period. ^{14}C recovered on the seventh day equals approximately one-twentieth that of the first day. Label excreted in the urine at times beyond 7 days is estimated to represent less than 1 per cent of the total dose excreted by the kidney.

Hourly pulmonary excretions of halothane for the first 8 hr were about half as great in the PBT-pretreated animals as in controls, decreasing nearly 100-fold during the initial 8-hr period studied in both instances (Fig. 3). While the excretion curve for controls follows apparent first-order kinetics with excretion decreasing by a factor of 2 every 70 min, in PBT-pretreated animals, the curve is biphasic with an initial, more rapidly decreasing component ($T_{1/2} = 42$ min) followed at 4 hr by a slower rate ($T_{1/2} = 105$ min).

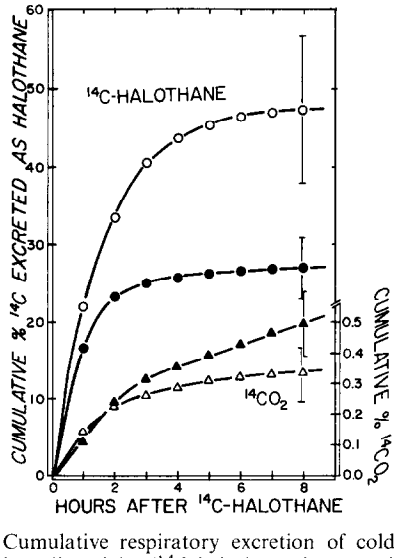

Fig. 1. Cumulative respiratory excretion of cold xylene-trappable radioactivity (^{14}C-halothane) in control (○) and PBT-pretreated animals (●) and of $^{14}CO_2$ in control (△) and PBT-pretreated (▲) animals during the first 8 hr after i.p. ^{14}C-1-halothane. Note difference in ordinates. Each point is the average of values for four animals. Brackets at 8 hr are S.E.M.

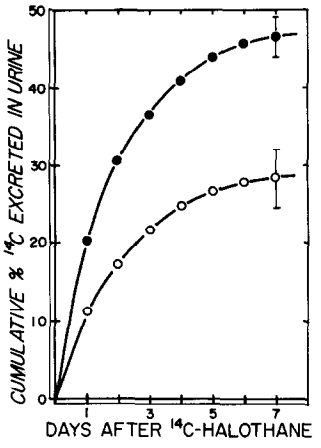

Fig. 2. Cumulative urinary excretion of nonvolatile ^{14}C from halothane in control (○) and PBT-pretreated (●) animals during the first 7 days after i.p. ^{14}C-1-halothane. Each point is the average of values for at least four animals. Brackets at 7 days are S.E.M.

The amounts excreted during this 8-hr period in both instances represent virtually all of the initial dose excreted as unmetabolized halothane by this route.

Hourly $^{14}CO_2$ excretions, initially several hundred-fold less than that of unmetabolized halothane, fell much more slowly, particularly in the PBT-pretreated animals (Fig. 3). In contrast, pulmonary excretion of $^{14}CO_2$ derived from intraperitoneal injection of $Na_2{}^{14}CO_4$ is more rapid, is virtually complete by 4 hr and follows first-order kinetics ($T_{1/2} = 29$ min). $^{14}CO_2$ entry into expired air should closely relate to total body CO_2 production [23]. Therefore, nearly constant rates of $^{14}CO_2$ excretion at times beyond 4 hr in PBT-pretreated animals are indicative of continuous breakdown of ^{14}C-1-halothane metabolites (Fig. 3).

Effects of prior anesthesia. A single 5-hr anesthesia with halothane was without effect on the metabolism of subsequent tracer doses of radioactive halothane, as determined by the urinary excretion of halothane metabolites (Table 1). Matched pairs of NaCl- and PBT-pretreated animals were given tracer doses of $^{14}CF_3CHBrCl$ on day 1 and metabolic products were collected to provide "control" values for each animal. All the animals were anesthetized with halothane 5 days later. Then 2 and 9 days after anesthesia, animals were given tracer doses of $^{14}CF_3CHBrCl$ and metabolic products were collected. Pulmonary and urinary excretion patterns were virtually identical to the pre-anesthesia values. In addition, no change was found in the relative amount excreted by either control or PBT-pretreated animals following re-anesthesia after a 2-week interval (Table 1). Throughout the experiment, the animals gained weight and appeared healthy.

The effects of repeated administration of anesthesia at 2-day intervals were also examined. PBT-pretreated animals were given a tracer dose of $^{14}CF_3CHBrCl$ on day 1 and urines were collected for 3 days to provide control values. On days 4, 6 and 8 of the experiment, the animals were anesthetized for 5 hr, and 48 hr after the last dose of anesthesia a second tracer dose was given and urine was

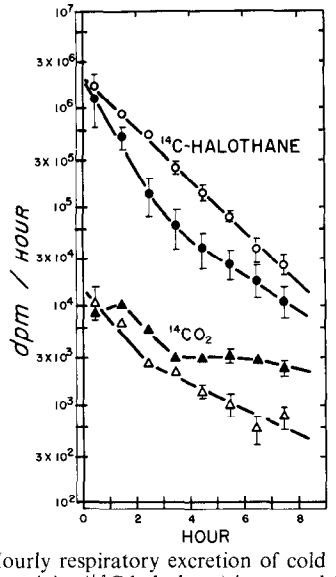

Fig. 3. Hourly respiratory excretion of cold xylene-trappable radioactivity (^{14}C-halothane) in control (○) and PBT-pretreated animals (●) and of $^{14}CO_2$ in control (△) and PBT-pretreated (▲) animals during the first 8 hr after ^{14}C-1-halothane. Each point is the average of values for four animals. Brackets are S.E.M. When brackets are not shown, S.E.M. is within the limits of the symbol.

Table 1. Effect of halothane anesthesia on ^{14}C-halothane excretion *in vivo**

Day	Schedule	Control (4)† Exhaled halothane‡	Control (4)† Metabolites in urine§	PBT (4)† Exhaled halothane‡	PBT (4)† Metabolites in urine§
		(dis/min × 10^6)			
1	Pre-anesthesia	4·0 ± 0·8	1·8 ± 0·3	2·1 ± 0·3‖	3·1 ± 0·2‖
8	2 Days post anesthesia	4·3 ± 0·4	2·3 ± 0·3	2·6 ± 0·5‖	3·7 ± 0·1‖
15	9 Days post anesthesia	5·3 ± 0·3	1·7 ± 0·1	2·2 ± 0·6‖	3·3 ± 0·5‖
22	2 Days post second anesthesia	4·2 ± 0·4	1·8 ± 0·2	2·4 ± 0·1‖	2·6 ± 0·2‖

* Anesthesia with 0·85% halothane for 5 hr. ^{14}C-halothane, 8 × 10^6 dis./min i.p.
† Number of animals.
‡ Trapped in cold zylene for 8 hr.
§ Collected for 7 days.
‖ $P < 0·05$ difference between control and PBT.

Table 2. Non volatile ^{14}C in liver 2 and 24 hr after ^{14}C-halothane*

Group†	Time (hr)	Total nonvolatile ^{14}C		Liver label distribution			
		Urine (dis./min × 10^4/24 hr)	Liver (dis./min × 10^4)	Residue	Mito	Micro	Cell sap
				(% total liver nonvolatile dis./min)			
Control (3)	2		17·7 ± 2·0	30 ± 3	3·4 ± 0·3	26 ± 1	39 ± 1
(5)	24	47 ± 3	5·7 ± 0·7‡	31 ± 1	8·1 ± 1·4‡	22 ± 2	43 ± 3
PBT (3)	2		20·2 ± 2·2	28 ± 7	1·6 ± 0·9	30 ± 3	42 ± 5
(5)	24	69 ± 5§	8·8 ± 0·2‡	33 ± 2	5·5 ± 0·8‡	22 ± 1‡	43 ± 1

* 6·7 × 10^6 dis./min i.p. at time 0; dose level, 5 μmoles halothane/kg animal.
† Number in parentheses is number of animal.
‡ $P < 0.05$ between 2-hr and 24-hr animals of a set.
§ $P < 0.005$ from corresponding control.

Table 3. Recovery of nonvolatile ^{14}C in chemical constituents after ^{14}C-halothane*

Group†	Time (hr)	Liver homogenate			Liver microsomes			Cell sap		
		Protein	Lipid	Acid sol	Protein	Lipid	Acid sol	Protein	Lipid	Acid sol
		(% total liver nonvolatile dis./min)			(% total liver nonvolatile dis./min)			(% total liver nonvolatile dis./min)		
Control (3)	2	30 ± 5	2·5 ± 0·1	36 ± 2	9·7 ± 0·7	1·1 ± 0·1	1·5 ± 0·1	0·9 ± 0·2	0·4 ± 0·3	25 ± 2
(5)	24	26 ± 4	1·2 ± 0·3‡	42 ± 3	9·3 ± 0·9	0·4 ± 0·1‡	1·8 ± 0·2	16·7 ± 2·5‡	ND§	31 ± 2
PBT (3)	2	26 ± 4	4·6 ± 0·5‖	41 ± 4	11·5 ± 2·3	2·2 ± 0·4‖	2·3 ± 0·3	1·5 ± 0·2	0·3 ± 0·2	26 ± 4
(5)	24	21 ± 2	4·2 ± 0·5	48 ± 3	9·4 ± 0·7	1·3 ± 0·2‡	2·6 ± 0·3	12·0 ± 1·0	ND§	32 ± 2

* 6·7 × 10^6 dis./min i.p. at time 0.
† Number in parentheses is number of animals.
‡ $P < 0.05$ between 2-hr and 24-hr animals of a set.
§ Not detected.
‖ $P < 0.005$ from corresponding control.

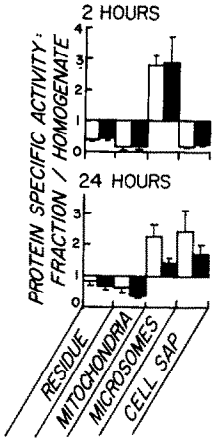

Fig. 4. The relative specific activity of protein in subcellular fractions of rat liver at 2 and 24 hr after i.p. ^{14}C-halothane in control (open bars) and PBT-pretreated (solid bars) animals. Each bar represents the average of values for at least four animals. Brackets are S.E.M.

collected for 3 days. The intervening doses of halothane anesthesia were without effect on the amount of halothane urinary metabolites recovered (2·3 ± 0·1 × 10^6 dis./min pre-anesthesia; 2·0 ± 0·3 × 10^6 dis./min post-anesthesia). When non-PBT-pretreated animals were used in a similar experiment, again the intervening doses of anesthesia did not alter the amount of urinary metabolites.

Hepatic binding. Although the urines of PBT-pretreated animals contain considerably more halothane-derived ^{14}C, their livers did not. Indeed when the increased weight of the liver in PBT-pretreated animals is taken into account, the label bound per g is equivalent at 2 hr (2·2 × 10^4 dis./min/g for controls compared to 2·0 × 10^4 dis./min/g for PBT-pretreated animals). As shown in Table 2, the radioactivity bound in the liver at 24 hr has decreased to one-third of that bound at 2 hr. At both 2 and 24 hr, the greatest portion of the total label bound is recovered in cell sap; microsomes and residue each contain between one-fifth and one-third the total label bound, and mitochondria the least.

As detailed in Table 3, the greatest portion of nonvolatile radioactivity recovered from liver is present in acid-soluble constituents, presumed to include trifluoroacetate and trifluoroethanol. One-third to one-quarter of the nonvolatile label in liver is recovered in protein and less than 5 per cent in lipid. Label incorporation into nucleic acids was not detected. At 2 hr, labeled protein is largely recovered in the residue and in microsomes, while labeled acid-soluble constituents are preferentially recovered in cell sap. PBT pretreatment seems to increase that proportion of label recovered in lipid. Between 2 and 24 hr, there is a striking increase in label in the cell sap protein in both controls and PBT-pretreated animals.

Microsomal proteins were preferentially labeled at 2 hr (Fig. 4) with specific activities (≅370 nmoles halothane metabolite bound/g of protein) at least 10-fold greater than those of residue, mitochondria or cell sap in both controls and PBT-pretreated animals. Between 2 and 24 hr, relative specific activities of cell sap proteins increased, becoming equivalent to those of microsomes. In contrast, selective labeling of microsomal lipids was not observed.

Effects of dose on halothane metabolism. As shown in Table 4, urinary excretion of halothane metabolites during the first 24 hr and recovery of nonvolatile

Table 4. ^{14}C-1-halothane metabolite in urine and liver at 24 hr*

Dose of halothane (μmoles/kg)†	^{14}C excreted in urine (μmoles/kg animal)	^{14}C bound in liver (μmoles/kg animal)	^{14}C bound in liver subfractions (μmoles/kg animal)	
			Protein	Microsomes
5 (9)	0·77 ± 0·08	0·097 ± 0·007	0·016 ± 0·001	0·023 ± 0·003
100 (6)	13·0 ± 1·6	1·87 ± 0·20	0·21 ± 0·03	0·49 ± 0·03
1,000 (6)	86·6 ± 10·6	11·2 ± 1·0	1·97 ± 0·13	2·89 ± 0·41
10,000 (4)	438 ± 67	54·4 ± 9·0	16·1 ± 1·7	15·0 ± 2·8

* ^{14}C-1-halothane, 7·8 × 10^6 dis./min i.p.
† Number in parentheses is number of animals. All animals were PBT-pretreated.

radioactivity in liver and its constituents at 24 hr increase with dose from the small dose (5 μmoles/kg) of undiluted $^{14}CF_3CHBrCl$ to the large dose (10,000 μmoles/kg), which narcotized animals and produced necrosis of free margins of the liver, fibrinous exudation and mild ascites. The relative portion of dose recovered in the urine and in liver decreased slightly with each successive increase in dose. Urinary excretion and total label bound in liver and microsomes increased approximately 600-fold and that recovered in liver protein and lipid increased 1000-fold over the 2000-fold dose range. In terms of per cent of total dose, 15 per cent of the smallest dose is excreted in the urine within the first 24 hr compared with 4 per cent after administration of the highest dose. Comparable values for label bound in liver are 1·94 and 0·54 per cent respectively.

Effect of CCl_4 pretreatment. A single small dose of CCl_4, a well known destroyer of MFOS activity *in vivo* [24], given 24 hr previously to PBT-pretreated animals strikingly decreases recovery of ^{14}C-1-halothane metabolites in liver components (Table 5). This dose of CCl_4 produces minimal histologic injury in PBT-pretreated animals [5].

DISCUSSION

Liver injury induced by halothane in the experimental animal occurs after a single anesthesia in phenobarbital-pretreated rats and is accompanied by a transient rise in lipid diene conjugation of microsomes and a more persistent loss of cytochrome P-450 [5, 25]. Basic to the design of this study was the assumption that halothane-induced liver injury is related quantitatively to the amount of halothane metabolized and of metabolite bound. Correlations between the amount of hepatotoxins bound and the extent of necrosis have been reported for $CHCl_3$ [26] and bromobenzene [27]. Indeed, increased binding of $^{14}CCl_4$ metabolites has been found in the areas of necrosis [28]. In preliminary studies we have found that PBT increases urinary excretion of labeled metabolites from $^{14}CCl_4$. In similarly pretreated animals, increasing doses of CCl_4 disproportionately increase label incorporation into lipid and microsomes. We therefore expected to find different metabolic patterns of halothane metabolism *in vivo* both for animals apparently "sensitized" to injury by PBT or by prior exposure to the anesthetic. While PBT strikingly enhances the metabolism of halothane, as determined by the increased excretion of metabolites in the urine, the amounts of metabolite bound in the liver are not increased. Prior halothane anesthesia apparently did not alter the metabolism *in vivo* of subsequently given labeled halothane as determined by the excretion patterns. This finding suggests that in the rat halothane does not induce its own metabolism. In fact, halothane anesthesia at short intervals actually seems to protect the animals from further anesthetic injury [5, 18].

Less than 1 per cent of the initial dose of halothane remains in the body as halothane 8 hr after its administration and the log linearity of the urinary excretion curve suggests relatively passive excretion of a fixed load of end stage metabolites, which have been reported to be chiefly trifluoroacetic acid [3, 29]. The apparent first-order excretion curves of halothane and its urinary metabolites in control and PBT-pretreated animals are indicative of a rapid metabolism, i.e. several hr, of the administered halocarbon. $^{14}CO_2$ and, for that matter, the amounts bound in the liver represent relatively minor proportions, 0·5 and 3 per cent respectively, of the total doses of halothane administered when compared to the 25–50 per cent

Table 5. Effect of CCl_4 pretreatment on urinary excretion and liver binding of $^{14}CF_3CHBrCl$ metabolites*

Group†	24-hr urine (μmoles halothane metabolite/kg)	Liver (μmoles halothane metabolite/kg)	Liver microsomes (μmoles halothane metabolite/kg)	Liver (μmoles halothane-^{14}C bound/kg liver)	
				Protein	Lipid
PBT (4)	0·91 ± 0·11	0·11 ± 0·01	0·029 ± 0·005	0·261 ± 0·050	0·025 ± 0·004
PBT-CCl_4 (5)	0·37 ± 0·09‡	0·06 ± 0·02‡	0·012 ± 0·005‡	0·151 ± 0·032‡	0·012 ± 0·002‡

* 260 μmoles CCl_4/kg *per os* 24 hr prior to halothane; $^{14}CF_3CHBrCl$, 7·4 × 10^6 dis./min (5·5 × μmoles halothane/kg) i.p.
† Number in parentheses is number of animals.
‡ $P < 0·05$.

of the total dose excreted in the urine. Pulmonary excretion of unmetabolized anesthetics and $^{14}CO_2$ and ^{14}C recovery from the urine were similar to prior reports for control [3, 19, 30] and PBT-pretreated animals [4]. However, patterns of $^{14}CO_2$ recovery were different in the PBT-pretreated animals, i.e. there was greater CO_2 production at later times. These more gradually declining or nearly constant $^{14}CO_2$ excretion patterns are indicative of continuing breakdown of the primary metabolites of the ^{14}C-1-halothane, presumably by oxidative defluorination [30].

The results of the study of metabolism *in vivo* presented here—that the overall metabolism of halothane by the animal is enhanced by PBT and inhibited by CCl_4, and that the early metabolic binding occurs preferentially in proteins derived from liver endoplasmic reticulum—support the hypothesis that the endoplasmic reticulum of the liver is the major intracellular site of halothane metabolism as well as the most likely site of injury by toxic metabolites of halothane. Mitochondria incorporate the least amount of label. CCl_4, which preferentially damages the enzyme systems of the endoplasmic reticulum, reduces hepatic binding and decreases excretion of halothane metabolites *in vivo*. Our finding of lack of enhancement of hepatic binding in PBT-pretreated animals *in vivo* is in accord with the study *in vitro* of Sipes *et al.* [31], but contrasts with the findings of other investigations *in vitro* [32–34].

Whether injury is related quantitatively to the amount of halothane metabolized, or to the amount of metabolite found in liver proteins and lipids, or to a relatively minor pathway of activation enhanceable under certain conditions remains to be seen. The initial step in halothane metabolism has been postulated to be its conversion to a free radical by an electron-capture reaction displacing its bromine [35], which could occur at one of several sites along the NADPH-P-450 electron transport system [34, 36]. The resulting trifluorochloroethyl radical would then enter into a series of reactions with its chemical environment. For example, as has been proposed for CCl_4 [36, 37], a halothane free radical-initiated lipid peroxidation, through the capture of a hydrogen atom from the methylene bridge of an unconjugated diene in unsaturated fatty acids of structural phospholipids, could result in the excretion of a halothane metabolite from the body as a gaseous metabolite. Resulting chemical changes in the lipid would not necessarily be associated with increased binding of halothane metabolites. Increased lipid-conjugated diene, which is evidence for the lipid peroxidation reaction [37], has been found only in anesthetized PBT-pretreated animals [5, 25]. Similarly, in a manner analogous to the action of CCl_4 [38], anesthesia with halothane may protect against a subsequent injury from re-anesthesia until such time as the responsiveness of the membrane to injury is restored. Unfortunately, our studies, which examined the major pathways of halothane metabolism as determined by urinary metabolites, CO_2 formation and hepatic binding, did not measure the conversion of halothane to trifluorochloroethane, trifluorobromoethane or trifluoroethane, all possible gaseous products of free radical electron-capture reactions of halothane.

Alternative toxic pathways for halothane must also be considered. Cohen [39] has reported the isolation of a condensation product between halothane metabolites and ethanolamine, tentatively identified as trifluoroacetylethanolamide, and has proposed that oxidized halothane metabolites may be converted to trifluoroacetaldehyde, which could then form Schiff bases with available primary amine groups within the cell. Factors other than those directly related to, or in addition to, halothane metabolism may also be important. For example, shock or relative ischemia, depletion of the liver glycogen, glutathione and other anti-oxidants may diminish the capacity of liver cells to convert halothane metabolites safely from a proximate toxin to a nontoxic derivative.

Acknowledgement—Halothane was supplied by Ayerst Laboratories.

REFERENCES

1. H. L. Price and R. D. Dripps, in *The Pharmacological Basis of Therapeutics* (Eds. L. S. Goodman and A. Gilman), 4th Edn., p. 86. MacMillan, London (1970).
2. R. A. Van Dyke and M. B. Chenoweth, *Biochem. Pharmac.* **14**, 603 (1965).
3. A. Stier, *Anesthesiology* **29**, 388 (1968).
4. R. A. Van Dyke, *J. Pharmac. exp. Ther.* **154**, 364 (1966).
5. E. S. Reynolds and M. T. Moslen, *Biochem. Pharmac.* **23**, 189 (1974).
6. R. J. Stenger and E. A. Johnson, *Proc. Soc. exp. Biol. Med.* **140**, 1319 (1972).
7. E. J. H. Ford, S. E. I. Adam and C. Gopinath, *J. comp. Path. Ther.* **82**, 355 (1972).
8. C. Gopinath and E. J. H. Ford, *J. Path. Bact.* **110**, 333 (1973).
9. J. P. Bunker, W. H. Forrest, Jr., F. Mosteller and L. D. Vandam, *The National Halothane Study*, U.S. Govt. Printing Office, Washington, D.C. (1969).
10. W. D. Reed and R. Williams, *Br. J. Anaesth.* **44**, 935 (1972).
11. W. H. W. Inman and W. W. Mushin, *Brit. med. J.* **1**, 5 (1974).
12. O. Almersjö, *Acta chir. scand.* **51**, (*Suppl.*) 135 (1972).
13. S. A. Fleming and W. G. C. Bearcroft, *Can. Anaesth. Soc. J.* **13**, 247 (1966).
14. J. C. Topham and S. Longshaw, *Anesthesiology* **37**, 311 (1972).
15. E. N. Cohen and E. N. Hood, *Anesthesiology* **31**, 553 (1969).
16. I. C. Geddes, *Laval méd.* **42**, 574 (1971).
17. H. C. Hughes, Jr. and C. M. Lang, *Anesthesiology* **36**, 466 (1972).
18. C. Gopinath, R. S. Jones and E. J. H. Ford, *J. Path. Bact.* **102**, 107 (1970).
19. R. A. Van Dyke, M. B. Chenoweth and A. Van Poznak, *Biochem. Pharmac.* **13**, 1239 (1964).
20. E. O'Malley, E. J. Conway and R. Fitzgerald, *Biochem. J.* **37**, 278 (1943).
21. G. H. Hogeboom, W. C. Schneider and G. E. Palade, *J. biol. Chem.* **172**, 619 (1948).
22. E. S. Reynolds, H. J. Ree and M. T. Moslen, *Lab. Invest.* **26**, 290 (1972).
23. F. G. Whitelaw, J. M. Brockway and R. S. Reid, *Q. Jl exp. Physiol.* **57**, 37 (1972).
24. J. V. Dingell and M. Heimberg, *Biochem. Pharmac.* **17**, 1269 (1968).
25. B. R. Brown, Jr., *Anesthesiology* **36**, 458 (1972).
26. K. F. Ilett, W. D. Reid, I. G. Sipes and G. Krishna, *Exp. molec. Path.* **19**, 215 (1973).

27. W. D. Reid and G. Krishna, *Exp. molec. Path.* **18**, 80 (1973).
28. A. L. Rogué and M. E. Fedorko, *J. Histochem. Cytochem.* **9**, 613 (1961).
29. D. A. Blake, J. Q. Barry and H. F. Cascorbi, *Anesthesiology* **36**, 152 (1972).
30. R. A. Van Dyke, M. B. Chenoweth and E. R. Larsen, *Nature, Lond.* **204**, 471 (1964).
31. I. G. Sipes, A. Sagalyn and B. R. Brown, *Fedn Proc.* **33**, 219 (1974).
32. L. C. Howard, D. R. Brown and D. A. Blake, *Soc. Tox. Abs.*, p. 153 (1974).
33. R. A. Van Dyke and C. L. Wood, *Anesthesiology* **38**, 328 (1973).
34. H. Uehleke, K. H. Hellmer and S. Tabarelli-Poplawski, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **299**, 39 (1973).
35. M. M. Atallah and I. C. Geddes, *Br. J. Anaesth.* **45**, 464 (1973).
36. E. S. Reynolds and M. T. Moslen, in *Free Radicals in Biology and Medicine* (Eds. H. Demopoulos and W. Pryor), Vol. II. Academic Press, New York, in press.
37. R. O. Recknagel, E. A. Glende, Jr., G. Ugazio, R. R. Koch and S. Srinivasan, *Israel J. med. Sci.* **10**, 301 (1974).
38. E. A. Glende, Jr., *Biochem. Pharmac.* **21**, 1697 (1972).
39. E. N. Cohen, *Anesthesiology* **35**, 194 (1971).