VOLATILE ANAESTHETIC METABOLISM AND ACUTE TOXICITY

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INTRODUCTION

Until the early 1960's, volatile anaesthetics were believed to be inert compounds not metabolised in the mammalian body. In 1964, using isotopically-labelled anaesthetics, it was shown that rats metabolise diethyl ether, chloroform and methoxyflurane to carbon dioxide, and halothane and methoxyflurane to chloride ion /1/.

Interest in the metabolism of volatile anaesthetics was greatly stimulated by the finding that renal dysfunction after methoxyflurane anaesthesia is caused by a metabolite of this agent /2, 3/. Simultaneously, a toxic metabolite was implicated in the hepatic necrosis caused by fluroxene and by chloroform in laboratory animals /4, 5, 101/ and soon afterwards halothane was reported to be hepatotoxic in rats which had been pre-treated with inducers of the hepatic mixed-function oxidase system /6, 7/. The last finding was of considerable interest as hepatic necrosis is a rare but serious complication of halothane anaesthesia in man.

The impact of studies such as these can be seen from the current trend toward the use and development of anaesthetics which undergo minimal metabolism. Clinical experience so far supports the enhanced safety of these agents with regard to organ toxicity.

Because volatile anaesthetics are always administered to man by inhalation, this is the preferred route of administration in animal studies. Intraperitoneal administration of neat halothane to rats results in inflammation of the peritoneum and consequent effects on the liver /8/. Determination of the proportion of anaesthetic metabolised presents a problem when the agent is administered by inhalation, as there is no quantity corresponding to the 'dose administered' of bolus administration. The extent of metabolism is therefore usually expressed relative to the net amount of anaesthetic absorbed during anaesthesia, or to the product of end alveolar concentration of anaesthetic and the duration of administration (MAC-hours*). Both of these methods provide a clinically useful index of metabolism for comparison of different agents. In addition, some workers have quantitated metabolism after parenteral administration of known amounts of radiolabelled anaesthetics.

* MAC – minimum alveolar concentration at which 50% of subjects do not respond to a surgical stimulus.

The following discussion will be limited to four clinically important volatile anaesthetics – methoxyflurane, enflurane, isoflurane and halothane. Although use of methoxyflurane has diminished in the last decade, it is included here because studies relating its metabolism to nephrotoxicity brought about a change in attitude of anaesthesiologists towards anaesthetic toxicity. Furthermore, because the nephrotoxic fluoride ion is formed (in lesser amounts) during metabolism of other volatile anaesthetics, the problem of renal toxicity is not exclusive to methoxyflurane.

The problems of malignant hyperpyrexia, mutagenicity and carcinogenicity of anaesthetics and their metabolites and of chronic effects of exposure to low levels of anaesthetics will not be discussed.

METHOXYFLURANE

Metabolism

In 1964, Van Dyke *et al.* /1/, using isotopically-labelled methoxyflurane, showed that in rats, the methyl group is converted to carbon dioxide and chloride ion is released. Holaday *et al.* (1970) /9/ reported conversion of methoxyflurane to carbon dioxide in humans and also identified the metabolites fluoride, dichloroacetic acid and methoxydifluoroacetic acid in urine. They suggested that oxalic acid might be a further metabolite of methoxyflurane and this was later confirmed /2/.

Liver, and to a lesser extent kidney, is primarily responsible for methoxyflurane dechlorination in the rat /10/. Enzyme activity is high in the liver 9,000 g supernatant, and moderate in the microsome fraction. A cytosolic enzyme, possibly a glutathione transferase, has been reported to catalyze release of fluoride from methoxyflurane *in vitro* /11, 12/, and may explain the high activity of the supernatant. Rabbit lung microsomes also metabolise methoxyflurane /13/.

Comparison of the rates of fluoride elimination from methoxyflurane and from perdeuteromethoxyflurane indicates that cleavage of a C-H bond is an essential step in methoxyflurane metabolism by rat liver microsomes /14/. Pathways of methoxyflurane biotransformation are summarized in Figure 1.

In humans, over 50% of net absorbed methoxyflurane is metabolised, mostly with the formation of fluoride, dichloroacetic acid and methoxydifluoroacetic acid /9/. Post-operative urinary oxalate ex-

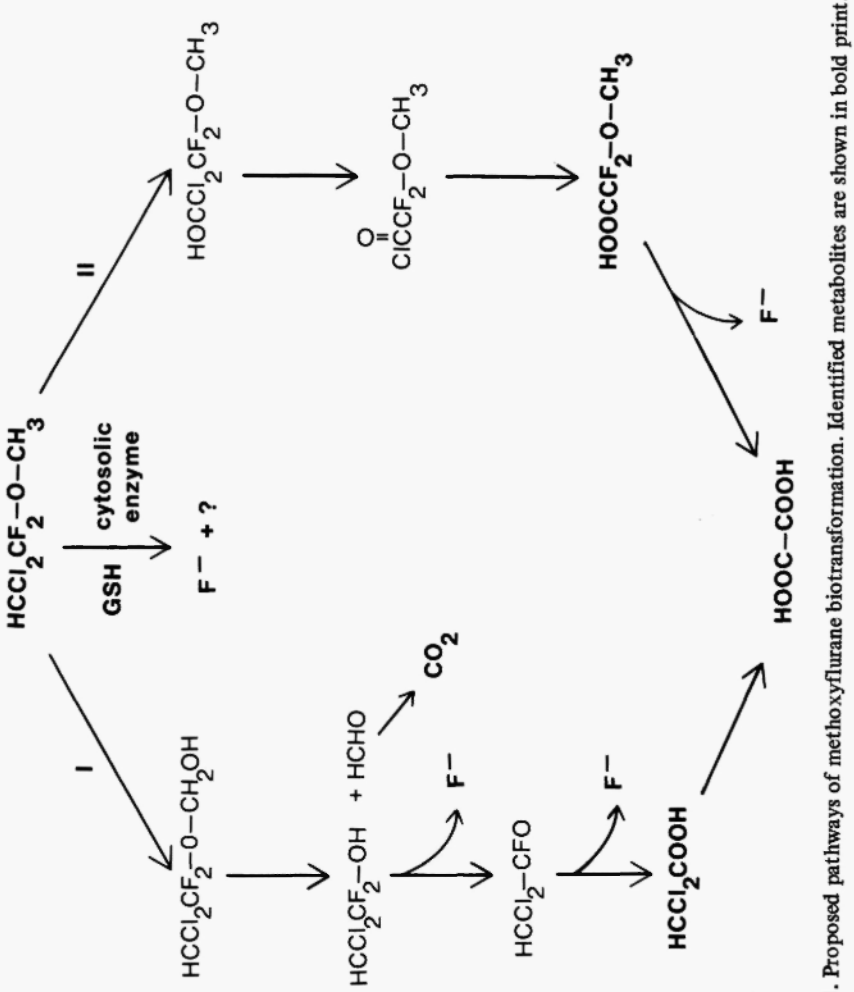


Fig. 1. Proposed pathways of methoxyflurane biotransformation. Identified metabolites are shown in bold print.

cretion represents only about 7% of absorbed methoxyflurane /15/. In these studies, only about 65% of the absorbed dose could be accounted for as exhaled and urinary fluorine-containing compounds, and extensive covalent binding of a reactive metabolite was postulated to account for the balance. Proposed metabolic intermediates include dichloroacetyl fluoride and methoxydifluoroacetyl chloride, both reactive compounds which could combine with tissue components. However, simple hydrolysis, as indicated in Figure 1, would almost certainly be the major pathway. The possibility that incomplete urine collection or impaired renal function contributed to the low recovery /16/ was ruled out /17/. Animal studies designed to quantitate the tissue distribution and extent of covalent binding of methoxyflurane metabolites may resolve the fate of the missing fluorine.

Renal Toxicity

In 1966, sixteen cases of post-operative renal dysfunction, characterized by diuresis and poor response to a vasopressin challenge, were reported in a group of 94 patients who had received methoxyflurane /18/. Despite the high incidence of toxic effects observed in this study; defects in its design /19/ as well as widespread acceptance of the safety of methoxyflurane left most anesthesiologists unconvinced that the results were applicable to the general patient population. Another early report of 20 cases of renal dysfunction in 180 patients given methoxyflurane /20/ also made little impression. Over the next five years, a number of reports of renal toxicity associated with methoxyflurane appeared, but it was not until 1971 that Mazze *et al.* /21/ reported the results of a randomized, prospective study which left no doubt as to the nephrotoxicity of this anaesthetic.

A report in 1970 had described a case of methoxyflurane nephrotoxicity associated with high levels of fluoride in serum and urine /22/. Subsequently, peak serum levels of both fluoride and oxalate were found to correlate with renal dysfunction in patients receiving methoxyflurane /2/. It was pointed out that the observed symptoms were unlikely to be caused by oxalic acid, but indirect evidence indicated that fluoride could be responsible for the polyuric renal insufficiency. The observation of calcium oxalate deposition in the kidneys of patients suffering renal failure after methoxyflurane anaesthesia /23, 24/ suggested the possibility that this was a contributing factor to the renal lesion. That fluoride is primarily responsible for methoxyflurane neph-

rotoxicity was demonstrated in an animal model /3/. In Fischer 344 rats, administration of fluoride, in an amount sufficient to simulate the urinary fluoride excretion following a nephrotoxic dose of methoxyflurane, produced polyuric renal failure and morphological changes similar to those seen after methoxyflurane. On the basis of the same dose of methoxyflurane, injection of a stoichiometric equivalent quantity of oxalic acid produced no abnormalities. Ten times this dose of oxalic acid resulted in anuric renal failure and morphological changes unlike those due to methoxyflurane (Figure 2). Thus, it appears that oxalic acid does not play a major role in the polyuric renal failure caused by methoxyflurane. It is, however, possible that calcium oxalate deposition may contribute to the lesion, especially in the case of the less common post-methoxyflurane anuric renal failure /25/.

Hollenberg *et al.* /26/, for example, described three cases of irreversible acute oliguric renal failure after methoxyflurane in which oxalate deposition was prominent. A case of generalized oxalosis after methoxyflurane has also been reported /27/. The patient had pre-existing renal insufficiency, and phenobarbitone had been administered preoperatively. Methoxyflurane-nitrous oxide was administered for about three hours. At autopsy, calcium oxalate crystals were found in the kidney, thyroid, bronchus, heart and retina. Increased metabolism and renal insufficiency may have led to high plasma oxalate levels in this patient.

Factors Predisposing to Methoxyflurane Nephrotoxicity

Dose

A dose-response relationship exists in methoxyflurane nephrotoxicity /25/. Parameters indicative of renal dysfunction correlate with dose of methoxyflurane (MAC-hours). Figure 3 shows the relationship between dose of methoxyflurane, peak serum fluoride concentration and nephrotoxicity for 18 patients. Nephrotoxicity was observed at peak serum fluoride levels above 50 μM, but it should be born in mind that this peak level is only an indicator of total kidney exposure (concentration x time) to fluoride.

Genetic Factors

Although Figure 3 indicates a strong dose-response relationship, considerable individual variation is apparent in both peak serum fluo-

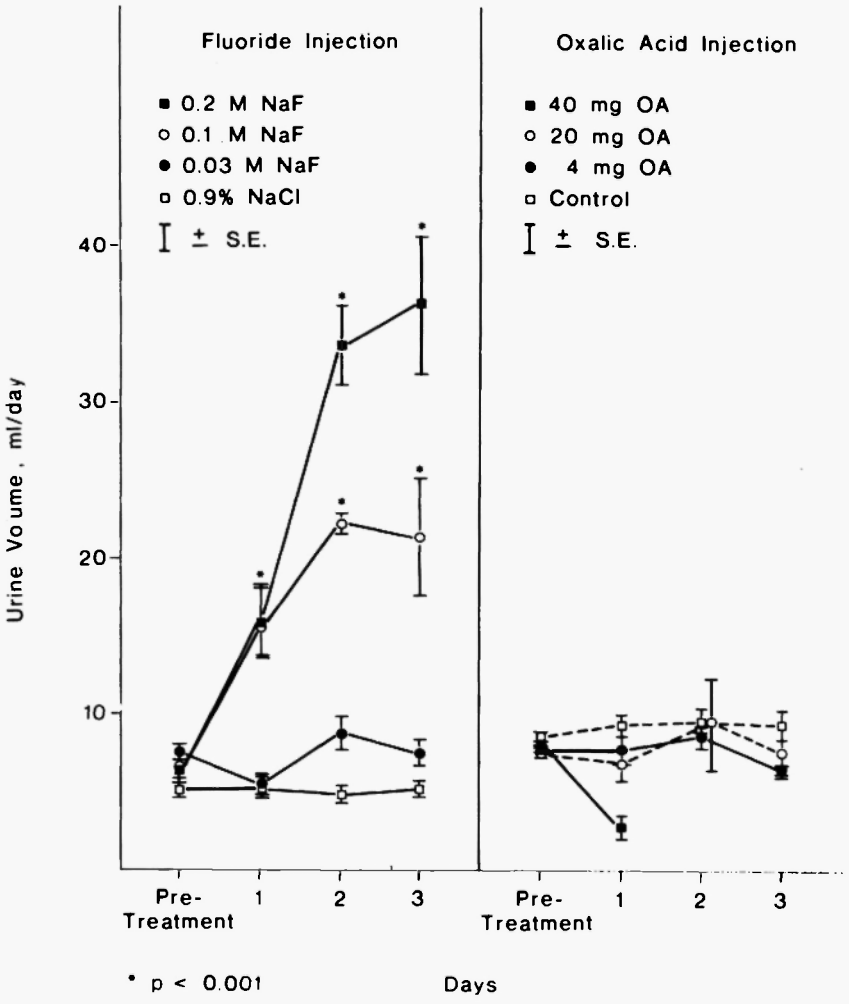
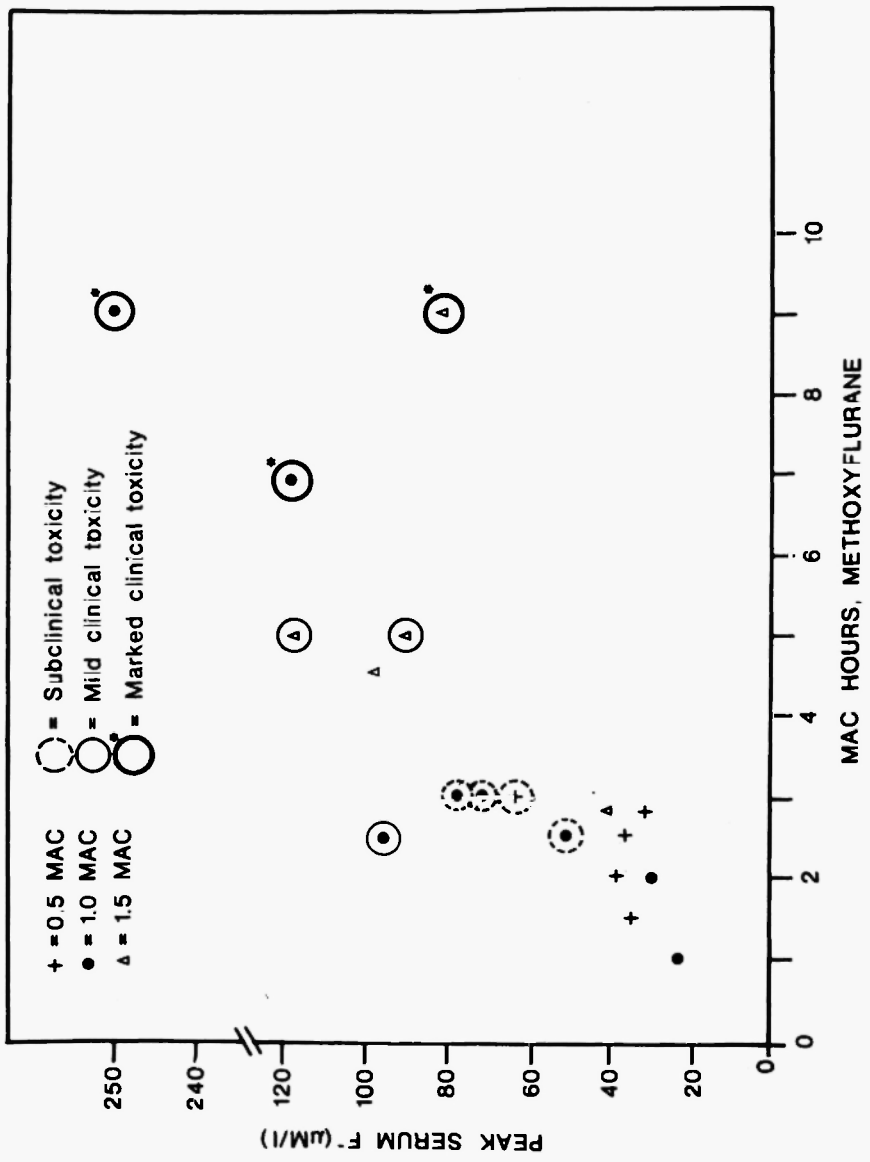


Fig. 2. Etiology of methoxyflurane nephrotoxicity. Intraperitoneal injection of methoxyflurane metabolites inorganic fluoride (NaF) and oxalic acid (OA) in amounts sufficient to simulate the urinary metabolite excretion following nephrotoxic doses of methoxyflurane. Dose related polyuria followed NaF injection but not injection of an equivalent amount of OA. Treatment with the highest dose of OA resulted in oliguria. *(Reproduced with permission from Cousins, M.J., Mazze, R.I., Kosek, J.C., Hitt, B.A. and Love, K.V.: The etiology of methoxyflurane nephrotoxicity. J. Pharmacol. Exp. Ther., 190:530-541, 1974).*



ride concentration after a given dose of methoxyflurane and degree of nephrotoxicity observed at each fluoride level. This indicates individual variation in both extent of methoxyflurane metabolism and renal sensitivity to fluoride, presumably due in part to genetic factors. Animal experiments provide evidence of genetic differences in both metabolism and organ sensitivity. Of six inbred rat strains, only Fischer 344 rats developed renal toxicity after anaesthesia for 3 hours with 0.5% methoxyflurane /28/. The susceptibility of this strain was due to both a high rate of methoxyflurane biotransformation and a high renal sensitivity to fluoride.

Age

Peak serum fluoride levels in paediatric patients (mean age 10.2 years) were found to be lower than those reported in adults after similar exposure to methoxyflurane /29/. This could be due to lower rates of methoxyflurane biotransformation or to altered fluoride pharmacokinetics (increased deposition in bone). No cases of methoxyflurane nephrotoxicity have been reported in children.

Enzyme induction

Factors which increase methoxyflurane metabolism increase the risk of nephrotoxicity. Treatment of rats with the mixed-function oxidase inducer phenobarbitone increases methoxyflurane metabolism /3, 30/ and exacerbates nephrotoxicity /3/. Treatment with 3-methylcholanthrene, an inducer of cytochrome P-448, does not result in increased microsomal metabolism of this anaesthetic /31/. Administration of isoniazid, or other compounds containing the hydrazine moiety, to rats results in increased rates of microsomal metabolism of methoxyflurane and other halogenated ether anaesthetics to inorganic fluoride /32, 33/. Although the hepatic microsomal content of total cytochrome P-450 is not increased by isoniazid, a specific form of the cytochrome may be induced /32/.

Presumably, mixed-function oxidase induction with appropriate agents increases methoxyflurane metabolism in man, but only indirect evidence is available to support this hypothesis. In the case of generalized oxalosis after methoxyflurane described by Bullock and Albert /27/, although complicating factors were present, phenobarbitone may have resulted in increased methoxyflurane metabolism. Cases involving

other anaesthetics (see 'enflurane') suggest that the increased anaesthetic metabolism caused by isoniazid in rats may also occur in man.

Other Nephrotoxic Drugs

Administration of other nephrotoxic drugs increases the likelihood of methoxyflurane nephrotoxicity. Treatment of rats with both gentamicin and methoxyflurane results in greater renal impairment than does treatment with either drug alone /34/. These two drugs also exert a synergistic effect on the kidney in humans. Severity of methoxyflurane nephrotoxicity was apparently aggravated as a result of commencement of gentamicin therapy in the post-operative period /35/. Tetracycline, a potentially nephrotoxic drug, has also been implicated as an exacerbating factor in methoxyflurane renal failure /36, 37/.

Obesity

Young *et al.* /38/ reported that obese patients develop higher serum fluoride levels during and two hours after methoxyflurane anaesthesia than do non-obese patients. Although it might be postulated that fat may serve as a depot for methoxyflurane, resulting in prolonged metabolism and sustained fluoride levels, in this study obese patients did not have higher serum fluoride levels 1-3 days post-anaesthesia. However, the authors compared their data from obese patients with data reported elsewhere for non-obese patients. Caution was recommended in the administration of methoxyflurane to obese patients.

Hepatotoxicity

A number of cases of liver injury after methoxyflurane, on occasion associated with fluoride nephrotoxicity, have been reported /39, 40/. Patients were often obese and had received either methoxyflurane or halothane previously /40/. In cases of hepatitis following repeat exposure, the latent period was shortened /40, 41/. Previous exposures have usually, but not always /42, 43/, been followed by fever and jaundice.

The number of patients who develop hepatitis after methoxyflurane and halothane or in whom methoxyflurane hepatitis followed previous exposure to halothane seems large in view of the rarity of severe hepatotoxicity after either anaesthetic /40/. This suggests either a cross-sensitization between the two anaesthetics, or that the same factors

(such as obesity, genetic makeup) predispose certain individuals to hepatotoxic effects of both agents. It is possible that methoxyflurane causes hepatitis by a mechanism similar, at least in part, to that of halothane hepatitis.

Progress in our understanding of methoxyflurane hepatitis has been at a standstill, largely due to lack of an animal model. The limited studies which have been carried out have not demonstrated reproducible hepatotoxicity in animals /44, 45/.

ENFLURANE

Conclusive proof of methoxyflurane nephrotoxicity in 1974 and emerging evidence of halothane hepatotoxicity has resulted in a sharp increase in the clinical use of enflurane, with a corresponding decrease in halothane use.

Metabolism

Enflurane is metabolised in man to fluoride ion, difluoromethoxydifluoroacetic acid and an unidentified acidic metabolite /46, 47, 48, 49/. The predicted products of oxidation at the difluoromethyl group, chlorofluoroacetic acid /48/ and oxalic acid /47/, have not been detected /47, 49/. Rat liver microsomes form the same metabolites as do humans and, like humans, do not appear to attack the difluoromethyl group /50, 51/. As in the case of methoxyflurane, the oxidative dehalogenation of enflurane involves initial attack at a C-H bond /51/. A proposed pathway for enflurane biotransformation is shown in Figure 4.

Enflurane metabolism is approximately one twentieth that of methoxyflurane and one tenth that of halothane. In a study in humans, about 83% of administered enflurane was exhaled unchanged and 2.4% recovered as urinary metabolites /46/. The incomplete recovery was probably due to underestimation of exhaled enflurane.

Renal Toxicity

Due to its minimal metabolism and rapid excretion, serum fluoride levels after enflurane are lower than those observed after methoxyflurane anaesthesia. After 2.7 MAC-hours of enflurane, serum fluoride concentration peaked at 22 μM 4 hours post-anaesthesia /47/ (Figure 5). However, some individuals may metabolise enflurane much more

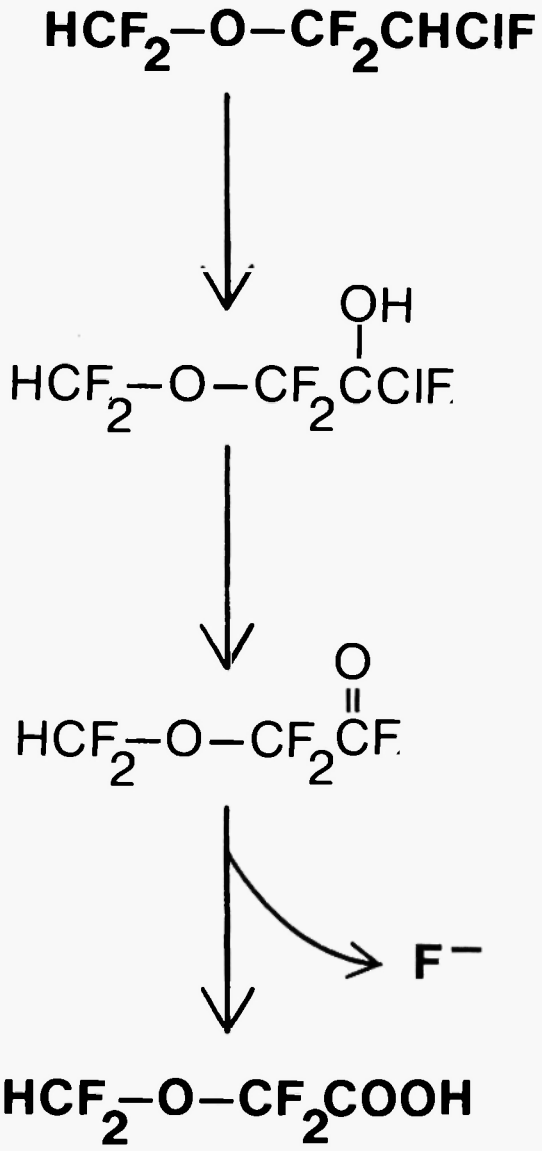


Fig. 4. Proposed pathway of enflurane biotransformation. Identified metabolites are shown in bold print.

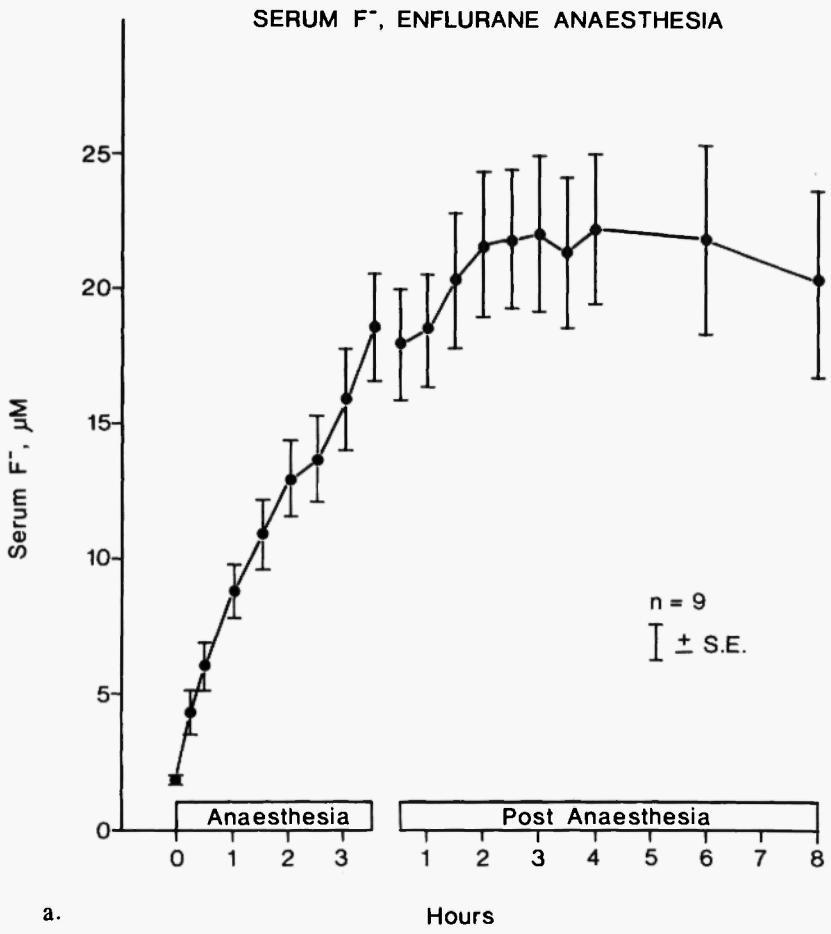


Fig. 5. Enflurane metabolism to serum inorganic fluoride (F^-) in man. (a) During anaesthesia enflurane metabolism increased rapidly with peak F^- of 22 μM in the early post-anaesthetic period.

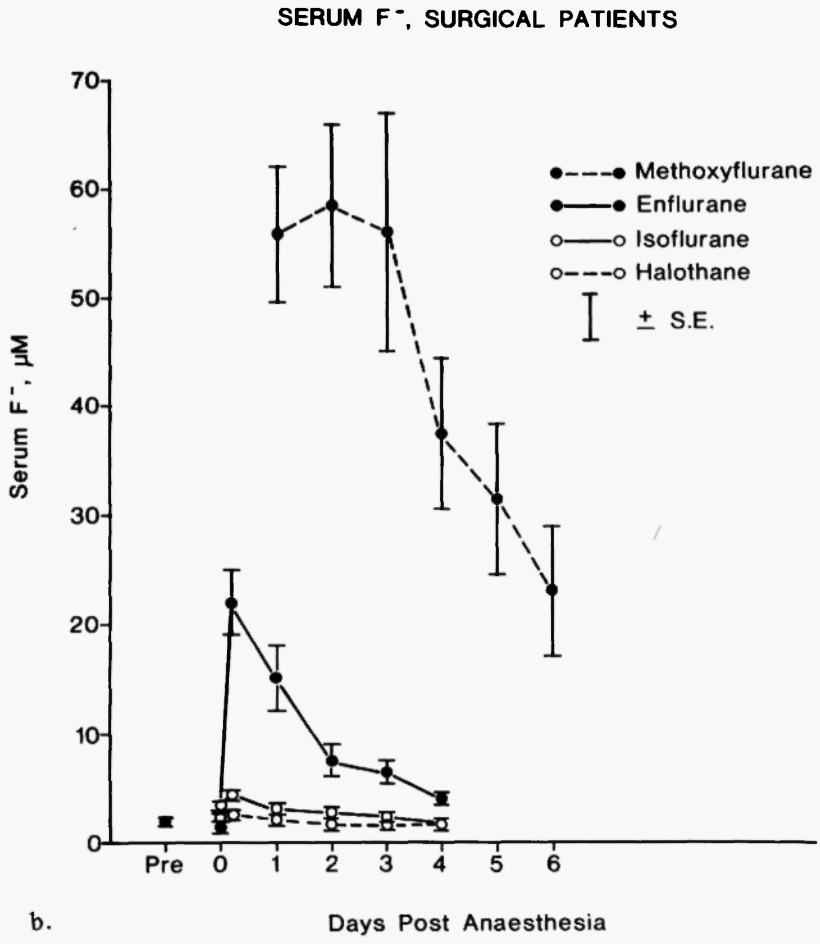


Fig. 5. Enflurane metabolism to serum inorganic fluoride (F^-) in man. (b) Following enflurane anaesthesia serum F^- peaked much earlier and declined to baseline much more rapidly compared to similar doses of methoxyflurane. Insignificant serum F^- concentrations followed similar doses of isoflurane and halothane. *(Reproduced with permission from Cousins, M.J., Greenstein, L.R., Hitt, B.A. and Mazze, R.I.: Metabolism and renal effects of enflurane in man. Anesthesiology, 44:44-53, 1976).*

extensively. In a reported case of post-operative renal failure, serum fluoride concentration was 93.6 μM on the second day after six hours of 1% enflurane (total dose = 3.5 MAC-hours) /52/. Increased enflurane metabolism and impaired fluoride excretion may have contributed to this high level. Clearly, where potentially exacerbating factors, such as prolonged anaesthesia, administration of other nephrotoxic drugs, enzyme induction or obesity are present, the possibility of fluoride nephrotoxicity must be considered.

Enflurane Dose

The effect of prolonged enflurane anaesthesia on renal function has been studied in rats and man. In Fischer 344 rats, 6–10 hours of 2.5% enflurane resulted in renal dysfunction similar to that in another group receiving 1.5 hours of 0.25% methoxyflurane /50/. Peak serum fluoride levels were similar in the two groups.

Renal effects of enflurane in man are dose-dependent. In patients receiving a mean of 2.7 MAC-hours of enflurane, renal function was not impaired 24–48 hours post-operatively /47/. Blood chemistry of volunteers receiving 9.6 MAC-hours of enflurane indicated minimal changes in renal function at 24 hours and 5 days /53/. However, in a similar study in which renal function was assessed by maximum urine osmolarity in response to vasopressin, impairment was observed 24 hours but not 5 days post-anaesthesia /54/.

Other Nephrotoxic Drugs

As is the case with methoxyflurane, administration of the nephrotoxin gentamicin to rats can exacerbate the renal effects of enflurane. Fischer 344 rats receiving gentamicin, 25 mg/kg twice daily for nine days, followed by 1 MAC enflurane for 6 hours, exhibit more pronounced morphological changes in the kidney and greater urine volumes than do animals receiving either gentamicin or enflurane alone /55/. With lower doses of gentamicin (5 mg/kg/day for 15 days) and enflurane (2% for 2 hours), no synergistic effect is observed in rats with surgically induced renal insufficiency /56/. Rats with surgically induced kidney damage, but not receiving gentamicin, develop only mild and reversible changes in renal function after 6 hours of 2% enflurane (20 days earlier, these animals had received 2 hours of 2% enflurane) /57/. The latter changes are probably not due to fluoride, as animals receiving 1% halothane instead of enflurane reacted in a similar manner.

Evidence relating to a combined effect of enflurane and nephrotoxic drugs in humans is lacking, but this could be due to caution in the use of enflurane in patients thought to be at risk. Enflurane anaesthesia has, however, been associated with renal dysfunction in several patients with pre-existing renal disease /52, 58, 59/.

Enzyme Induction

Treatment of rats with the mixed-function oxidase inducing agent, phenobarbitone, results in increased metabolism of enflurane by the hepatic microsomal fraction *in vitro* /60, 61, 62/. However, phenobarbitone treatment does not affect enflurane metabolism or nephrotoxicity *in vivo* /50, 63/. Similarly, in a group of 102 surgical patients classified according to drug history, no differences were found in peak serum fluoride concentration after enflurane between control patients and those taking miscellaneous drugs, ethanol or drugs (phenobarbitone, phenytoin) known to cause enzyme induction /64/.

As mentioned earlier, the rate of metabolism of halogenated ether anaesthetics is increased in hepatic microsomes prepared from isoniazid-treated rats /32/. This effect is also observed *in vivo*. Isoniazid-treated Fischer 344 rats exposed to 2% enflurane became polyuric and excreted increased fluoride relative to similarly exposed rats not treated with isoniazid /65/. Isoniazid appears to have a similar effect in man. In one study of enflurane metabolism, one patient had an unusually high serum fluoride concentration (106 μM after 3.8 MAC-hours of enflurane) /47/. This patient had been taking several drugs, including isoniazid. Fish *et al.* /33/ refer to additional cases of elevated fluoride levels in patients receiving isoniazid prior to enflurane anaesthesia. As the hydrazine moiety is responsible for the inducing effect /33/, enflurane should be used with caution in patients taking drugs containing this group.

Obesity

Obesity also contributes to elevated fluoride levels after anaesthesia. Cousins *et al.* /47/ reported an obese patient (patient #7) who had a peak serum fluoride concentration of 52 μM after 4 MAC-hours of enflurane. After similar exposures to enflurane, serum fluoride concentrations were higher in obese than in non-obese patients (28 *versus* 17 μM) /66, 67/. The rate of increase of serum fluoride levels during and after 2 MAC-hours of enflurane in obese patients (5.5 μM/hour)

was twice that in a non-obese group (2.5 μM/hour). These differences were apparently due to more rapid biotransformation of enflurane in the obese group.

Overall, there appears to be little risk of fluoride nephrotoxicity after moderate doses of enflurane in the absence of risk factors. Reports of enflurane nephrotoxicity are rare, despite the fact that risk factors (obesity, isoniazid therapy, prolonged anaesthesia) are presumably present in a large number of enflurane administrations. Enflurane is excreted more rapidly than methoxyflurane, and this results in fluoride levels peaking earlier and returning to baseline levels more quickly in comparison to methoxyflurane /47, 50/. Thus, for the same peak serum fluoride concentration, exposure of the kidney to fluoride is more prolonged after methoxyflurane than after enflurane (see Figure 5b).

Hepatotoxicity

A number of cases of possible enflurane hepatotoxicity have been reported. In some cases, previous anaesthesia with halothane /68, 69, 70/ or enflurane /71, 72/ had been uneventful. Only in one case was the patient reported as being obese /70/. In none of the cases was the diagnosis unequivocal. That the chemically related anaesthetics, halothane, methoxyflurane and fluroxene, can be hepatotoxic may have predisposed some anaesthetists to incriminate enflurane where other causes could not be found. Hepatotoxicity of these other agents indicates that careful scrutiny of the possible role of enflurane in unexplained hepatitis is warranted, but should not be used as evidence against this anaesthetic.

Two prospective studies of liver function after multiple halothane or enflurane anaesthesias have been reported. In one, 49 black females undergoing radium therapy for carcinoma of the cervix received enflurane on 1-3 occasions /73/. No significant changes in liver function tests were observed. In a second study in which 66 patients received enflurane 2-5 times, minimal changes in liver function were observed, but the frequency did not increase with increasing numbers of anaesthesias /74/.

In volunteers, 9.6 MAC-hours of enflurane caused minimal changes in liver function, as assessed by blood chemistry 1 and 5 days after anaesthesia /53/. In addition, enflurane (0.6–2% for 55-230 minutes)

did not affect antipyrine half-life or clearance (4 and 8 days post-anaesthesia) in a group of surgical patients /75/.

The failure to detect evidence of liver injury in prospective studies indicates that enflurane is not hepatotoxic to the general population. If enflurane was indeed the causative agent in reported cases of 'enflurane hepatitis', then these patients must have been rendered susceptible by factors (environmental, genetic, pre-existing disease) which are at present unidentified.

No animal studies have reported acute hepatotoxicity due to enflurane. Administration of enflurane to LE (control and phenobarbitone induced) /76/, Sprague-Dawley (phenobarbitone treated, anaesthetized in 12% or 100% oxygen) /77/ or Fischer 344 (phenobarbitone treated, anaesthetized in 14% oxygen) /78/ rats does not produce liver injury. Only further research and clinical experience will establish whether enflurane hepatitis is a clinical entity, and if so, what factors predispose to it.

Other Effects

Anaesthesia with enflurane, especially at high concentrations, often causes CNS excitation, manifested as muscular movements and increased electroencephalograph activity /79, 80, 81, 82/. These effects are, however, completely reversible.

ISOFLURANE

Although synthesised soon after enflurane, clinical introduction of isoflurane was delayed until 1981. This was partly due to initial difficulty in purifying the drug but also to subsequently disproven studies reporting carcinogenicity.

Metabolism

Isoflurane, an isomer of enflurane, is metabolised to inorganic fluoride and trifluoroacetic acid in both rats and man /83, 84, 85/. As studies with enflurane have shown the difluoromethyl group to be metabolically stable, biotransformation of isoflurane is likely to proceed by initial attack at the chlorine-bearing carbon. A proposed pathway for isoflurane metabolism is shown in Figure 6.

Isoflurane is metabolised to a lesser extent than other clinically

used volatile anaesthetics. In humans, almost the entire dose is exhaled unchanged, less than 0.2% is excreted as urinary metabolites /86/. Fischer 344 rats also metabolise isoflurane minimally, serum fluoride concentration reaching only 6.5 μM after 4 MAC-hours and 7.3 μM after 15 MAC-hours of isoflurane /83/ (preanaesthetic values are 2-3 μM).

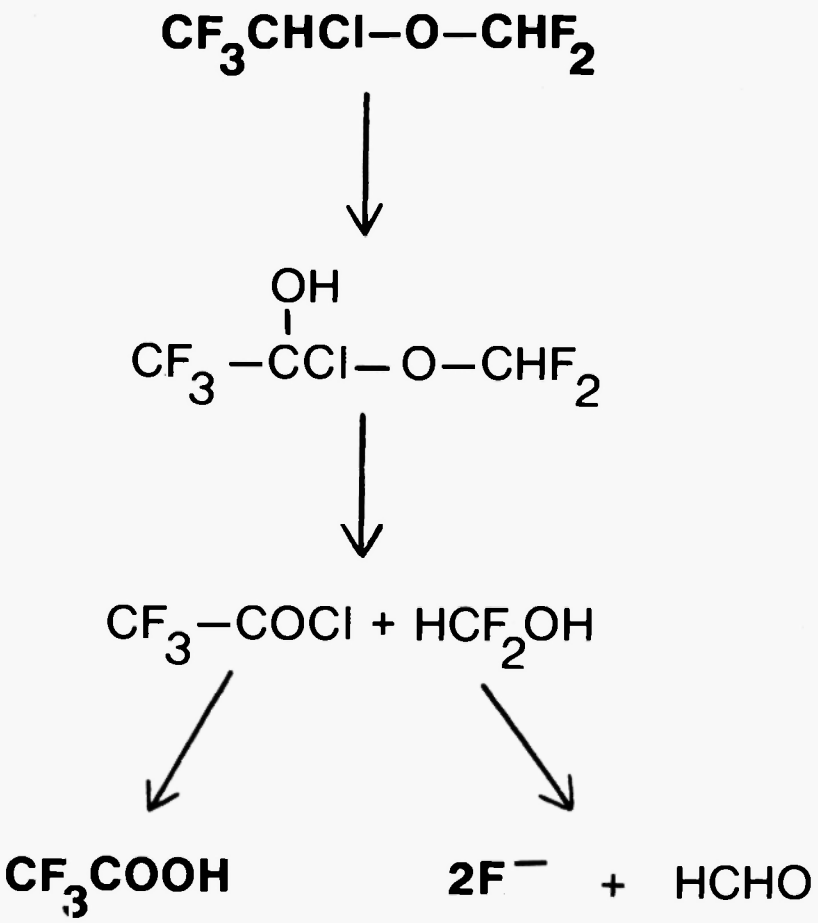


Fig. 6. Proposed pathway of isoflurane biotransformation. Identified metabolites are shown in bold print.

Renal Toxicity

Due to the minimal extent of metabolism of isoflurane, fluoride nephrotoxicity has not been associated with its use in man /84/ or rats /83/. In humans, peak serum fluoride concentrations average 4.4 μM (twice the preanaesthetic value), well below the nephrotoxic threshold, after 1.2-5.3 MAC-hours of isoflurane /84/ (see Figure 5).

Metabolism of isoflurane is increased in hepatic microsomes from rats treated with phenobarbitone /60, 87/ or isoniazid /32/. Phenobarbitone induction does not increase the rate of isoflurane metabolism *in vivo* /87/. The effects of isoniazid have not been studied *in vivo,* but based on results of *in vitro* studies /32/ it is unlikely that induction of the mixed-function oxidase system could result in sufficient stimulation of isoflurane metabolism to cause fluoride nephrotoxicity.

Hepatotoxicity

No clinical reports associating isoflurane anaesthesia with liver damage have appeared.

In the few reported animal studies of hepatic effects of isoflurane anaesthesia, there was no evidence of liver injury. Anaesthesia of phenobarbitone-treated male Sprague Dawley rats with isoflurane (1.4%) in oxygen followed by a hypoxic environment does not result in liver damage /77/. Similarly, phenobarbitone-treated male Fischer 344 rats anaesthetised for 2 hours with isoflurane (1.5%) in 14% oxygen do not exhibit increased serum alanine aminotransferase or morphological changes 24 hours post-anaesthesia /78/.

HALOTHANE

Metabolism

Halothane is metabolised to chloride /1/, bromide /88/, fluoride /89/, carbon dioxide /90, 91/, trifluoroacetic acid /88/, 2-chloro-1,1,1-trifluoroethane and 2-chloro-1,1-difluoroethylene /92/, N-trifluoroacetyl-2-aminoethanol and N-acetyl-S-(2-bromo-2-chloro-1,1-difluoroethyl)-cysteine /93/. In man, urinary bromide excretion indicates that about 20% of net absorbed halothane is metabolised /88/. Many pathways have been proposed to explain the observed (and some unobserved) metabolites. Figure 7 shows a possible scheme.

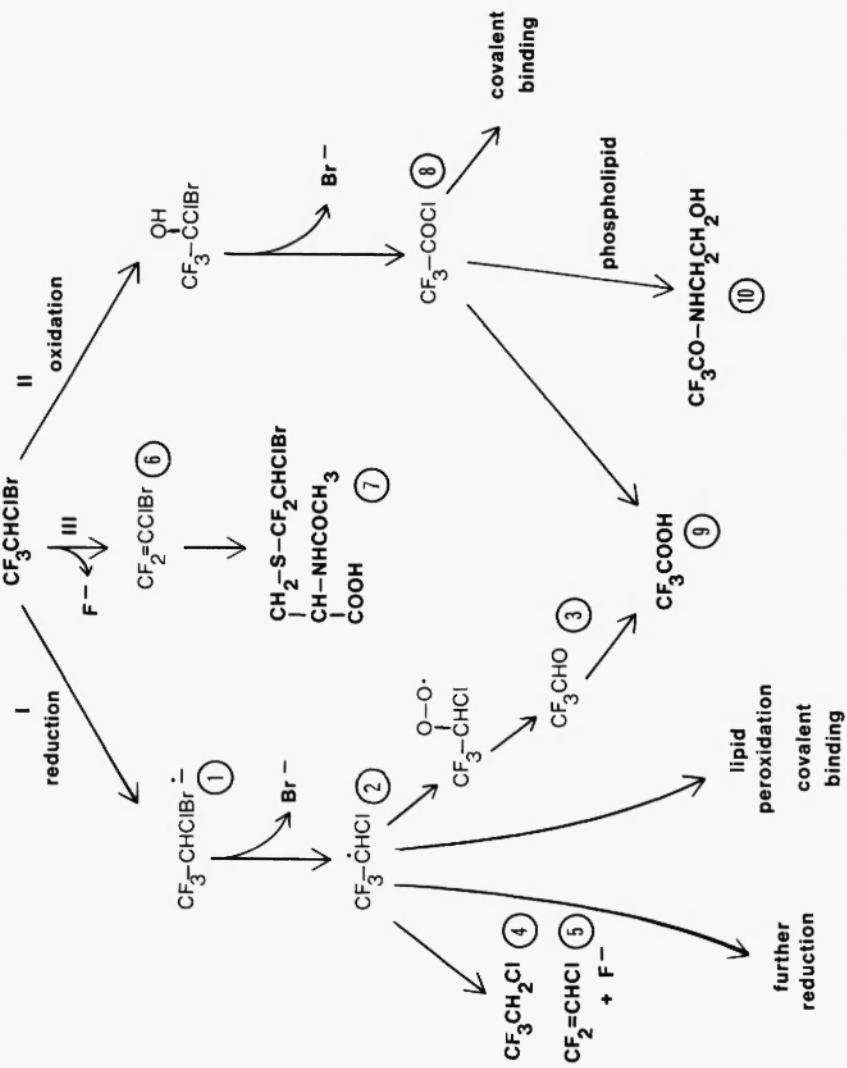


Fig. 7. Proposed pathways of halothane biotransformation. Identified metabolites are shown in bold print.

A knowledge of halothane biotransformation is vital to an understanding of the possible mechanisms of its hepatotoxicity. Therefore, the pathways shown in Figure 7 will be discussed in detail.

(i) Reductive Pathway (Pathway I)

Also called the 'non-oxygen dependent' pathway /94/, this is a true reduction (according to the mechanism shown in Figure 7) as it involves initial acceptance of an electron by halothane. The intermediate radical anion *1* is shown to illustrate a possible mechanism of the reduction. The bromide ion leaves simultaneously with acceptance of the electron /95/. The resulting neutral free radical *2* can follow one of several pathways.

(a) Reaction with oxygen

Carbon-centred free radicals have a high affinity for molecular oxygen and react with it spontaneously to form peroxy radicals. Presumably this reaction is possible for radical *2*. The end product would be trifluoroacetic acid, a metabolite usually referred to in the literature as a product of the oxidative pathway only. The obligatory intermediate trifluoroacetaldehyde (*3*) and a likely further product, trifluoroethanol, have not been detected. This pathway is therefore probably quantitatively unimportant as a source of trifluoroacetic acid, in keeping with the view that reductive metabolism of halothane accounts for only a very small fraction of the dose. The significance of this pathway is that it would give oxygen a role in the scavenging of free radical *2*, and hence in controlling tne amount of this radical available for covalent binding or the formation of reduced metabolites.

(b) Further reduction

It has been postulated that radical *2* may be further reduced by cytochrome P-450 to form a carbanion /96/. This carbanion could eliminate a fluoride ion to form 2-chloro-1,1-difluoroethylene *(5)* or a chloride ion to form a carbene, or could abstract a proton from water or other cellular component to form 2-chloro-1,1,1-trifluoroethane (*4*). *In vitro* isotopic studies, however, indicate that the additional hydrogen in 2-chloro-1,1,1-trifluoroethane does not arise from water or other sources of exchangeable hydrogen /97/. Therefore if a carbanion is formed, it must not be released as such from cytochrome P-450. Evidence has been described indicating that a carbene-cytochrome P-450 complex is formed by the interaction of halothane with rat

liver microsomes *in vitro* /98, 99/ or by perfusion of isolated rat livers with a halothane-containing medium /99/. No evidence is available linking carbene formation with the hepatotoxic effects of halothane.

(c) Covalent binding and lipid peroxidation

Free radical *2* could covalently bind to tissue components (for example by addition across double bonds in unsaturated lipids) or initiate lipid peroxidation (by abstracting a hydrogen atom from unsaturated lipids). Halothane does stimulate lipid peroxidation in phenobarbitone /100, 101/ or Aroclor 1254 /102/ treated rats, and covalent binding has been reported (see later).

(d) Production of reduced metabolites

Hydrogen abstraction by radical *2* gives rise to 2-chloro-1,1,1-trifluoroethane *(4)* /97/. The hydrogen atom is probably derived from lipid. The mechanism of formation of 2-chloro-1,1-difluoroethylene *(5)* is unknown, but its level in exhaled breath of halothane-anaesthetised rats closely parallels the level of 2-chloro-1,1,1-trifluoroethane /96, 103/, suggesting a common precursor.

Both 2-chloro-1,1,1-trifluoroethane and 2-chloro-1,1-difluoroethylene are present in the exhaled breath of halothane-anaesthetised rabbits /92/, rats /96/ and man /96, 104/.

(ii) Oxidative Pathway (Pathway II)

The oxidative pathway is believed to proceed via the reactive intermediate trifluoroacetyl chloride *(8)* /93/. This compound can react with water to form trifluoroacetic acid, or can covalently bind to nucleophilic groups on cellular molecules. A human urinary metabolite of halothane, N-trifluoroacetyl-2-aminoethanol *(10)*, presumably arising by hydrolysis of trifluoroacetyl-phosphatidylethanolamine, provides evidence of covalent binding of *8* /93/.

(iii) Glutathione Conjugation (Pathway III)

The mercapturic acid, *(7)*, a human urinary metabolite of halothane, is believed to arise by conjugation of 1-bromo-1-chloro-2,2-difluoroethylene *(6)* with glutathione, followed by removal of glutamic acid and glycine and acetylation /93/. The reaction of *6* with glutathione (perhaps catalysed by the glutathione transferases) is likely, as an analogue, 1,1-dichloro-2,2-difluoroethylene, reacts with nucleophiles to

form derivatives substituted at the fluorine-bearing carbon /105/. However, *6* has not been identified as a metabolite of halothane. It is formed by interaction of halothane with warm, moist soda lime, and has been detected in closed-circuit anaesthetic systems where soda lime is used /104/. It is not found when a Bain circuit (non-rebreathing) is used.

In vivo, 1-bromo-1-chloro-2,2-difluoroethylene *(6)* could be formed by removal of a proton from halothane by cytochrome P-450 (as proposed by Ullrich and Schnabel /106/), followed by loss of a fluoride ion. This pathway is neither an oxidation nor a reduction, though *6* is often described as arising via a reductive pathway. Because *(6)* has a high blood:gas partition coefficient /107/ and is rapidly metabolised /107, 108/ small amounts could be formed in the body without detectable levels in the expired breath.

Renal Toxicity

Biotransformation of halothane results in the formation of insufficient fluoride to cause kidney damage (See Figure 5). Twenty-four hour urinary excretion of fluoride is not significantly increased after 1.8 MAC-hours of halothane /84/.

No reports of nephrotoxicity following halothane have been published.

Hepatotoxicity

The rare, severe liver injury caused by halothane in humans is believed to occur only when a number of different factors (genetic and environmental) interact /109/. To further complicate matters, different mechanisms may be operative in different patients.

The development of animal models has shed much light on certain individual factors which may contribute to halothane hepatitis. Perhaps the most important and fundamental information gained from animal studies is that under appropriate conditions, halothane is a hepatotoxin able to cause injury in 100% of exposed animals /96/. This knowledge permits the hypothesis that under some circumstances, halothane anaesthesia also directly causes liver damage in humans.

Role of Biotransformation in Halothane Hepatotoxicity in Animal Models

The liver is the major site of metabolism of xenobiotics. Although the end products of biotransformation are usually less toxic than the parent molecule, the reactions involved in this detoxication often proceed via short-lived electrophilic intermediates (epoxides, free radicals, carbonium ions) which are potentially damaging to cellular enzymes and organelles. Hence, it is not surprising that liver damage due to the majority of hepatotoxins is caused not by the parent compound, but by a metabolite. Substantial experimental evidence indicates that this is the case with halothane.

Hepatotoxicity of End Products of Halothane Biotransformation

Pretreatment of rats with the hepatic mixed-function oxidase inducing agents, phenobarbitone or polychlorinated biphenyls, renders them susceptible to halothane hepatotoxicity /6, 7, 96, 102, 110, 111, 112/, while inhibitors (SKF 525 A, metyrapone) of this enzyme system protect rats against the damage /112/. This indicates that metabolites of halothane may mediate the toxic effect, and so some effort has been made to establish whether any of the known end-products of halothane biotransformation are hepatotoxic. Although 2-chloro-1,1,1-trifluoroethane *(4)* has been reported to be hepatotoxic in rats /113/, there is some doubt as to the true identity of the material used in this study. Furthermore, the vehicle (propylene glycol) and route of administration (i.v. into the portal vein) do not permit extrapolation of this result to liver injury caused by halothane anaesthesia. In a more recent study, inhalation of the volatile halothane metabolites 2-chloro-1,1,1-trifluoroethane (*4*, 2.65%) or 2-chloro-1,1-difluoroethylene (*5*, 0.35%) in 14% oxygen for 2 hours did not result in increased serum alanine aminotransferase activity or morphological changes in the livers of phenobarbitone-treated rats /114/.

Administration of the major organic metabolite of halothane, trifluoroacetate *(9)* to rats via their drinking water, caused changes in liver size and metabolism similar to those observed after exposure to halothane /115/. In this study, hepatocellular necrosis after trifluoroacetate or halothane was not reported. Blake *et al.* /116/ observed changes in liver/body weight ratios of rats when trifluoroacetate was administered in drinking water, but not when i.p. administration was used. They assigned these changes to rejection of trifluoroacetate-con-

taining water by the rats. Doses of up to 5 g/kg of sodium trifluoroacetate, administered by i.p. injection, did not elicit toxic effects in mice /117/.

It has not, however, been established that exogenously administered trifluoroacetate enters hepatocytes. When formed intracellularly during metabolism of halothane, trifluoroacetate may have access to targets not reached when i.p. or oral administration is used. Studies of the toxicity of non-ionic trifluoroacetate precursors should reveal whether this is the case. Compounds such as 2,2,2-trifluoroethanol and trifluoroacetaldehyde will enter hepatocytes and there be partially converted to trifluoroacetate; trifluoroacetaldehyde has a relatively low toxicity in mice but trifluoroethanol is toxic, apparently due to its intracellular conversion to trifluoroacetate /117/. However, even after a lethal dose of trifluoroethanol, no histologic change were observed in the livers of mice or dogs. Thus, it seems unlikely that halothane hepatotoxicity could be mediated by trifluoroacetate.

Hepatotoxicity and Reactive Intermediates of Halothane Metabolism

Because the major end products of halothane metabolism do not appear to be hepatotoxic, attention has focussed on the short-lived intermediates. Evidence points to intermediates of reductive metabolism (Pathway I) being responsible for halothane hepatotoxicity in animal models.

It has been suggested that the proposed intermediate in Pathway III, 1-bromo 1-chloro-2,2-difluoroethylene *(6)*, may alkylate cellular macromolecules /93/. However, this intermediate is believed to be rapidly metabolised by conjugation with glutathione, and depletion of glutathione does not result in hepatotoxicity in phenobarbitone-treated rats anaesthetised with halothane in air /101/ or in untreated rats anaesthetised with halothane in 8% oxygen /110/. Furthermore, mice exposed to 1-bromo-1-chloro-2,2-difluoroethylene (0.006 – 0.5%) developed renal lesions, but liver damage was not described /118/.

Intermediates of the oxidative metabolism of halothane (Pathway II) do not appear to be involved in hepatotoxicity. Replacement of the hydrogen atom of halothane by deuterium does not affect the extent of its reductive metabolism (assessed by fluoride, 2-chloro-1,1,1-trifluoroethane and 2-chloro-1,1-difluoroethylene levels) in a rat model, but formation of bromide and trifluoroacetate (metabolites arising mainly from the oxidative pathway) is reduced /119/. Both halothane

and deuterohalothane produce similar degrees of liver injury, implicating reductive rather than oxidative metabolism as a cause of hepatotoxicity.

A substantial amount of additional evidence links reductive metabolism with hepatotoxicity. Hypoxia is an important factor contributing to halothane associated liver injury in rat models /96, 110, 111, 112, 120/, and results in increased exhalation /96/ and blood levels /114/ of reduced volatile metabolites and increased plasma fluoride levels /110/. Low intracellular oxygen concentration could result in less effective scavenging of radical *2* by molecular oxygen, or could permit halothane to compete more effectively with oxygen for cytochrome P-450, resulting in increased reductive metabolism.

Covalent binding studies also demonstrate the presence of higher levels of reactive intermediates under hypoxic conditions. Studies using ^{14}C, ^{3}H or ^{36}Cl halothane have shown that covalent binding, especially to phospholipids, is increased under hypoxic conditions /121, 122, 123, 124/. Only the metabolite binding to lipid retains the chlorine atom /125/. Although a number of different intermediates may be involved in covalent binding, results of the above studies are consistent with the view that free radical *2* binds substantially to lipid, while trifluoroacetyl chloride *(8)* binds to both lipid and protein (with loss of the chlorine).

Attention is currently focussed on free radical *2* as a possible toxic intermediate of reductive metabolism. Spin-trapping studies have confirmed the presence of free radicals in the livers of halothane anaesthetised rats /78, 126/. The structure of these radicals has not been determined, but *2* is consistent with the results of covalent binding studies, the formation of 2-chloro-1,1,1-trifluoroethane /97/ and the lability of the C-Br bond. Although the 1-bromo-1-chloro-2,2,2-trifluoroethyl radical has been proposed as a metabolic intermediate of halothane /93, 125, 127, 128/, this is not the structure of the radical trapped in rat liver /78/.

There is no direct evidence that free radicals mediate halothane hepatotoxicity. Indeed, incubation of not only halothane, but also of enflurane, methoxyflurane or fluroxene with rat liver microsomes results in the formation of free radicals /129/, although it is not clear whether these radicals are derived from the anaesthetics or from endogenous compounds such as unsaturated lipids. It is unlikely that such *in vitro* studies are relevant to metabolism in the whole animal. *In vivo,*

no free radicals are found in the livers of rats anaesthetised with enflurane or isoflurane, agents not hepatotoxic in the animal model used /78/. In contrast free radicals are trapped following administration of halothane or carbon tetrachloride, drugs which are hepatotoxic under the conditions of administration /78/.
cause hepatocellular damage. Covalent binding or abstraction of hydrogen atoms from essential macromolecules, such as enzymes in the endoplasmic reticulum, could result in irreparable damage and/or loss of some vital cellular function. Initiation of lipid peroxidation could result in disruption of membranes and release of toxic lipid breakdown products. Another possible mechanism is covalent binding of free radicals to tissue macromolecules resulting in the formation of antigens.

Applicability of Animal Models of Halothane Hepatotoxicity to Man

The usefulness of animal models of human toxicity is largely governed by the degree of similarity of the mechanisms of toxicity in the model and in man, and differences in metabolism and physiology. For example, in a number of species of animals fluroxene produces fatal hepatic necrosis /5, 130/, but as humans metabolise fluroxene in a different manner, these results cannot be extrapolated to man /131, 132/.

As detailed mechanisms of halothane hepatoxicity are unknown in either animals or man, assessment of models must be made on the basis of the way in which various factors influence the response of the liver. As the most widely used animal model is presently the phenobarbitone-induced, hypoxic rat model, only this will be discussed here.

Comparison of the model with man can be made on the basis of the following criteria:

(i) Routes and extent of halothane biotransformation.
(ii) Role of metabolism in hepatotoxicity.
(iii) Genetic susceptibility.
(iv) Sex differences.
(v) Multiple exposures.
(vi) Role of immune response.
(vii) Pathology of the lesion.
(viii) Other factors.

(i) Routes and Extent of Halothane Biotransformation

Major pathways of halothane biotransformation are the same in male Fischer 344 rats and man /96/. Rats exhale higher levels of the volatile metabolites, especially under conditions of mixed-function oxidase induction and hypoxia (Figure 8), and achieve higher serum bromide levels. After i.p. administration of a sub-anaesthetic dose of (^{14}C)-halothane, male Charles River rats excrete about 25% of the dose in the urine in one week /91/. After phenobarbitone pretreatment, urinary excretion of metabolites is approximately doubled. Intravenous administration of isotopically labelled halothane to humans results in excretion of 10-25% in the urine in 1-2 weeks /133/.

Two human urinary metabolites of halothane, N-trifluoroacetyl-2-aminoethanol *(10)* and N-acetyl-S-(2-bromo-2-chloro-1,1-difluoroethyl)-cysteine *(7)*, have not been reported in rats. However, as noted previously, halothane metabolites do react with phospholipid in rat liver and this is the proposed mechanism of formation of *10*.

Overall, halothane biotransformation in rat and man is sufficiently similar to justify the use of the rat as a model. The use of induced, hypoxic rats in which metabolism is increased is probably unavoidable – metabolism must be stimulated in order to produce hepatic injury in all treated animals.

(ii) Role of Metabolism in Hepatotoxicity

As indicated above, overwhelming evidence indicates that halothane biotransformation plays a crucial role in production of hepatic injury in the rat hypoxic model. There is no evidence to date that this is the case in humans. One case of possible halothane hepatitis has been reported in which the patient may have been exposed to polychlorinated biphenyls /134/ – potent inducing agents known to promote halothane hepatotoxicity in rats /7/. However, *known* exposure to enzyme inducing agents is not a common feature of reported cases.

The halothane molecule is too inert to covalently bind to cellular components, and too small to act as an antigen. The assumption that reactive metabolites mediate the injury, either directly or indirectly, is essential to proposed mechanisms of halothane hepatitis. Proof of this assumption is very difficult. Studies of halothane biotransformation in patients suffering halothane hepatitis may provide some evidence, but by the time jaundice develops (1-16 days) and a diagnosis is made, re-

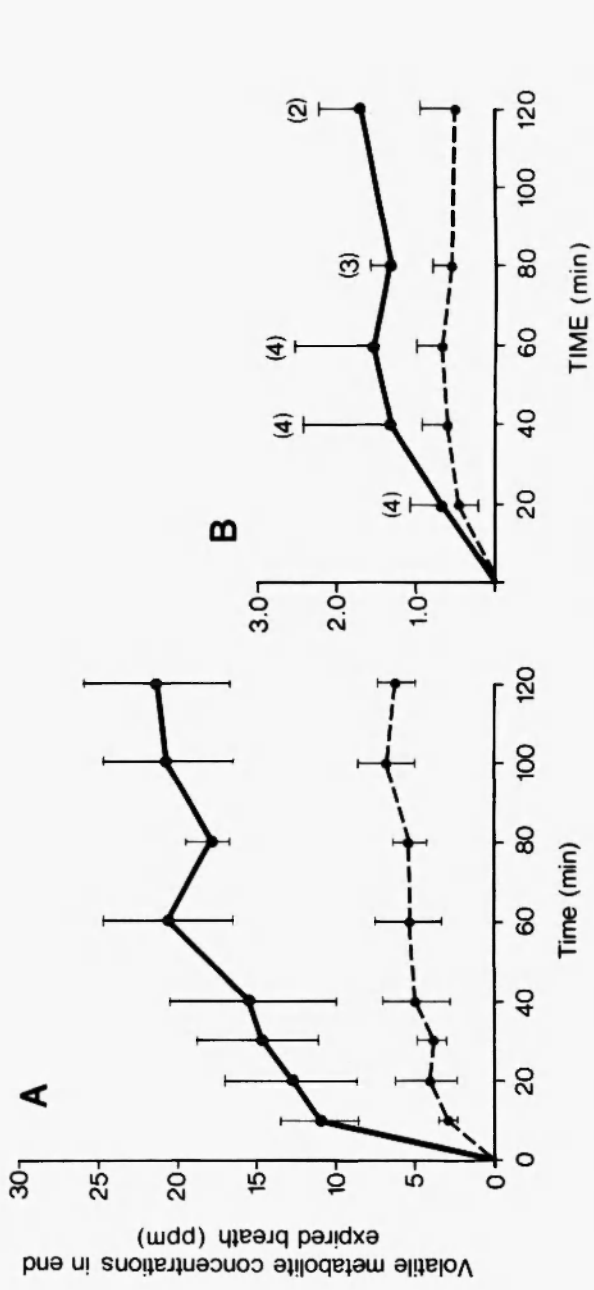


Fig. 8. Exhalation of volatile metabolites during halothane anaesthesia of rats and man. A. Concentrations of 2-chloro-1,1,1-trifluoroethane (●—●) and 2-chloro-1,1-difluoroethylene (●- -●) in end-expired breath of phenobarbitone-induced Fischer 344 rats anaestetized with 1% halothane in 14% oxygen in nitrogen. B. Concentrations of 2-chloro-1,1,1-trifluoroethane (●—●) and 2-chloro-1,1-difluoroethylene (●- -●) in end-expired breath from patients anaesthetized with 1% halothane in 99% oxygen. Figures in parentheses are the number of patients studied at each time point. *(Reproduced with permission from Gourlay, G.K., Adams, J.F., Cousins, M.J. and Sharp, J.H.: Time-course of formation of volatile reductive metabolites of halothane in humans and an animal model. Br. J. Anaesth., 53:331-336, 1980).*

duced halothane metabolites are likely to be at levels which are difficult to detect.

(iii) Genetic Susceptibility

The rarity of halothane hepatitis has given rise to speculation that certain individuals are genetically susceptible. A recent report of halothane hepatitis in three pairs of closely related women lends considerable weight to this hypothesis /135/. In the hypoxic model, different inbred rat strains vary in severity of liver damage /136/. Differences in both halothane metabolism and susceptibility of the liver to injury are involved (Figure 9).

Current opinion supports the idea that genetic predisposition is an important, but not the only, factor in determining an individual's likelihood of developing halothane hepatitis /94/.

(iv) Sex differences

Halothane hepatitis is more common in human females than in males /137/. In the rat hypoxic model, however, female Sprague-Dawley rats are more resistant than males /112/. Female rats metabolise foreign compounds more slowly than do males. This difference, not observed in humans, may explain the resistance of female rats to halothane hepatotoxicity.

(v) Multiple Exposures

In a large proportion of reported cases of halothane hepatitis, halothane has been administered on a previous occasion. The first exposure was sometimes followed by unexplained fever or jaundice. Clinicians may consider the diagnosis of halothane hepatitis to be more certain if previous exposures have occurred, and this would result in over-reporting of these cases relative to those occurring after a first exposure. Nevertheless, the proportion of cases of severe liver damage involving multiple exposures, and the frequency of mild liver damage following multiple halothane exposures /74/, are so high that repeat exposure, especially at short intervals, is universally accepted as being a risk factor /138/.

There is little evidence that multiple exposures of rats to halothane result in more extensive liver damage than does a single exposure. Bromsulphthalein retention by perfused livers from rats exposed to

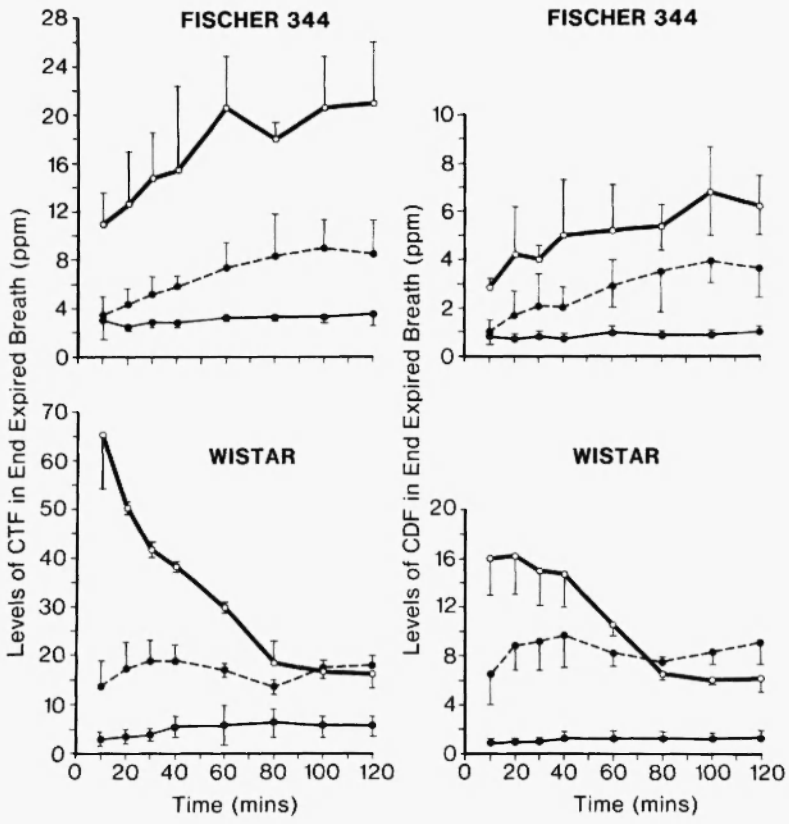


Fig. 9. Time course of formation of 2-chloro-1,1-difluoroethylene (CDF) and 2-chloro-1,1,1-trifluoroethane (CTF) in Fischer 344 and Wistar rats anaesthetized with halothane under various conditions. Exposure conditions and pretreatment are as follows: O—O phenobarbitone-pretreated, 1% halothane, 14% oxygen; ●- -● untreated, 1% halotnane, 14% oxygen; ●—● phenobarbitone-pretreated, 1% halothane, 21% oxygen. *(Reproduced with permission from Gourlay, G.K., Adams, J.F., Cousins, M.J. and Hall, Pauline: Genetic differences in reductive metabolism and hepatotoxicity of halothane in three rat strains. Anesthesiology, 55:96-103, 1981).*

halothane three times on alternate days, but not to diethyl ether three times or to halothane once, was increased /139/. The effect was significant at one, three and four weeks after exposure to halothane. This change is probably distinct from the hepatotoxicity due to halothane in the hypoxic model, as in this model, in most animals, recovery of the liver is complete in five days (as assessed by serum alanine aminotransferase activity and light microscopy) /140/.

Congestion of the liver was reported after exposure of rats to 0.5% halothane for 15 min/day, 6 days/week for three months /141/. Again, this observation is unrelated to halothane hepatotoxicity as similar exposure to 4% diethyl ether caused the same changes.

When rats are exposed to halothane under conditions leading to hepatocellular damage, a protective effect of previous exposure is observed. Anaesthesia of phenobarbitone-treated rats with 0.85% halothane for 5 hours produces foci of centrilobular necrosis on the posterior aspect of the liver /142/. When animals are exposed repeatedly under these conditions at 48 hour intervals, the extent of injury became progressively less, damage not usually being observed after the 4th–6th exposure. Impaired halothane metabolism after the first exposure, due to destruction of components of the hepatic mixed-function oxidase system, probably results in lowered susceptibility to halothane.

In general, rats react differently to humans to multiple halothane exposure. However, it is unclear whether, for purposes of comparison with humans, rats should be re-exposed before or after recovery of the liver has occurred. No work has been reported in which liver injury is induced in rats by halothane, but allowed to recover fully before a second exposure.

(vi) Role of Immune Response

The increased frequency and reduced latent period of liver damage after multiple halothane exposures suggests the possibility that an immune response could be involved in halothane hepatitis. Indeed, positive challenge tests with halothane have been carried out on two anaesthetists said to be allergic to halothane /143, 144/, but the conclusions of these studies have been severely criticised /145/.

One possible mechanism of halothane hepatitis involving the immune system assumes conversion of halothane to reactive intermediates. Covalent binding of reactive halothane metabolites to tissue macro-

molecules in the endoplasmic reticulum could result in the formation of potentially antigenic compounds. These could migrate to the plasma membrane /146/ and reaction with an antibody directed against the altered hepatocyte surfaces could result in hepatocellular necrosis.

Animal experiments confirm that halothane metabolites can act as haptens under certain circumstances. Trifluoroacetyl groups bound to protein can stimulate an immune response in guinea pigs and rabbits /147, 148, 149/. However, sensitization of guinea pigs to such antigens does not render them more susceptible to halothane hepatotoxicity than control animals /150/. Furthermore, extent of hepatic necrosis after halothane does not correlate with response to a skin test with trifluoroacetate conjugated to guinea pig albumin /151/. As discussed previously, covalent binding studies in rat liver suggest that the trifluoroacetyl group is the major group binding to protein, hence this is probably an appropriate hapten. It appears, therefore, that halothane hepatotoxicity in guinea pigs is not mediated by an immune response against trifluoroacetylated liver components.

Similarly, in the rat hypoxic model, an immune response is clearly not a factor contributing to liver damage. The absence of enhanced toxicity in rats after multiple exposures, and the rapid onset of damage (first evident at the end of a 2 hour exposure /140/) indicate that direct metabolite-mediated toxicity is responsible.

Recent studies have contributed toward understanding the role of the immune system in human halothane hepatitis. The sera of 7 out of 11 patients who had developed fulminant hepatic failure after halothane contained an antibody directed against the surface of hepatocytes isolated from halothane-exposed rabbits /152/. The antibody did not react with hepatocytes from an ether-anaesthetised rabbit, and was not present in the sera of patients with hepatitis due to drugs other than halothane or in the sera of healthy donors. In addition, the antibody was not present in the sera of patients who developed mild liver abnormalities after halothane /153/.

It is not clear whether this antibody causes liver damage or is an associated phenomenon /154, 155, 164/. Numerous studies have demonstrated covalent binding of halothane metabolites to components of the endoplasmic reticulum. Possibly antibodies form against these altered liver components only *after* damage to the cell has occurred. If so, then an immune response could occur secondary to direct toxicity, perhaps causing further deterioration of an otherwise reversible insult.

Fig. 10. Relationships between rat hypoxic model and human halothane hepatitis. In this scheme it is proposed that reactive intermediates, formed during halothane metabolism, can cause hepatocellular damage by either of two mechanisms. Direct toxicity results from damage to vital cellular enzymes or organelles, while covalent binding to macromolecules may give rise to antigens which may migrate to the cell surface. Antigens may also arise or be unmasked as a result of direct toxicity, without necessarily contributing to hepatic injury. Usefulness of animal models of halothane hepatitis is largely dependent on the relative importance of these two mechanisms in the model and in man.

A possible causative role for the antibody-antigen reaction in liver injury cannot be ruled out. That antibody was detected in only a proportion of severe halothane hepatitis patients and in none with mild damage, is consistent with two distinct mechanisms /153/ – immunologically-mediated necrosis and direct metabolite-mediated damage.

The rat hypoxic model for halothane hepatotoxicity does not involve an immune response and thus it may be considered only as a model for direct hepatotoxicity. This concept is illustrated in Figure 10.

(vii) Pathology of the Lesion

In the hypoxic induced rat model, hepatic necrosis has been reported in the region of the central vein, twenty-four hours after halothane administration /96/. One hundred percent of these animals show hydropic changes in hepatocytes in zone 3 (perivenular region) of the liver; when marked this change is termed ballooning degeneration (Figure 11). In addition all animals show varying amounts of liver cell

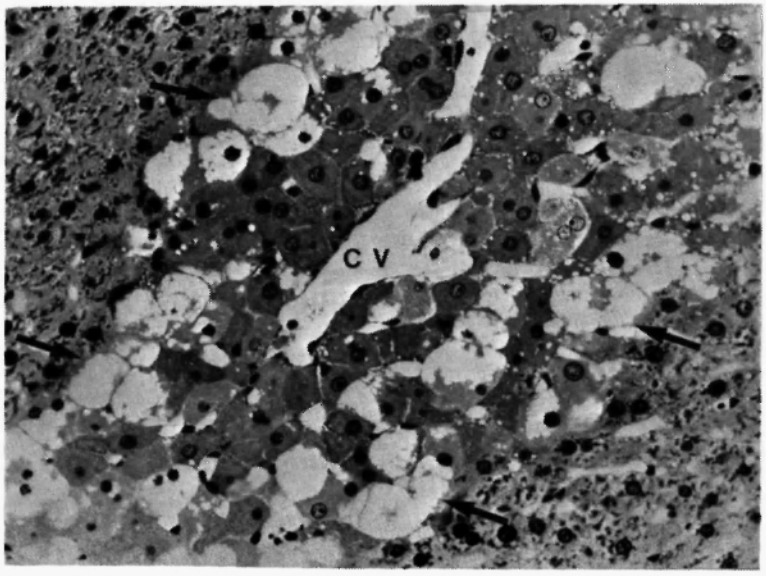


Fig. 11. Portion of a section of a liver biopsy taken from a hypoxic induced rat 24 hours after halothane anaesthesia. A central vein (CV) is surrounded by hepatocytes showing varying degrees of hydropic change. The arrows indicate some of the hepatocytes showing marked ballooning degeneration. Normal liver cells are present in the top left and lower right parts of this microphotograph. Haematoxylin and eosin X350.

necrosis and an associated inflammatory cell response. Features indicating repair and regeneration in the liver are not seen 24 hours after halothane exposure. Animal studies have mostly described hepatic damage with a short latent period following halothane whereas most human liver tissue has been examined several weeks after halothane anaesthesia /156/. It is hoped that our current study of the time course of halothane hepatitis in the hypoxic rat model /140/ will permit more meaningful comparisons with halothane hepatitis in man.

(viii) Other Factors

A large proportion of patients developing halothane hepatitis are obese /137/. This might be expected if toxicity was dose-related, as release of halothane stored in fat could result in prolonged exposure of the liver to toxic intermediates. However, dose of halothane appears not to be an important factor, as prolonged anaesthesia is not a feature of reported cases of halothane hepatitis. Obesity could result in increased metabolism of halothane; serum fluoride levels after halothane are higher in obese than in non-obese patients /38/. This indicates, in particular, increased metabolism by non-oxygen dependent pathways (Pathways I and III, Figure 7). It is also possible that the sensitivity of the liver may be increased in some obese patients, due, for example, to decreased oxygen delivery to the liver during anaesthesia. The effects of obesity in animal models have not been investigated.

Radiation therapy has also been associated with an increased incidence of liver dysfunction after halothane. Patients with carcinoma of the cervix, undergoing multiple halothane anaesthesias for radium insertion, had a high incidence of mild /157/ and severe /158/ liver damage. On the basis of these studies and the knowledge that γ-radiation breaks halothane down to the toxic dichlorohexafluorobutene, it has been suggested that repeated use of halothane is contra-indicated during radiation therapy /159/. A more recent study, however, found no association between halothane and liver injury in a patient group similar to those of the earlier studies /160/.

Mice exposed to low levels of both halothane and γ-radiation do not develop more severe liver damage than those exposed to halothane alone /161/. The evidence implicating radiation therapy as a contributing factor in halothane hepatitis is, at present, unconvincing.

PERSPECTIVE

As might be expected from their lack of affinity for specific macromolecules (enzymes, receptors), organ toxicity due to volatile anaesthetics is associated with their metabolism. The order of extent of biotransformation: methoxyflurane > halothane > enflurane > isoflurane, is the same as the order of frequency of organ-directed toxicity. In the belief that this trend will also be followed by other halogenated anaesthetics, development of new agents is aimed largely toward compounds undergoing minimal metabolism.

Genetic predisposition is of prime importance in determining whether an individual will develop organ toxicity when exposed to an anaesthetic. In the case of methoxyflurane nephrotoxicity, both extent of metabolism and kidney sensitivity to fluoride show individual variation. Whether genetic susceptibility to halothane hepatitis is due mainly to more extensive metabolism, greater susceptibility of the liver, or both, is unknown. Individuals do metabolise halothane at different rates, but for this to be solely causative of responses varying from no liver damage to fatal massive necrosis would imply an enormous range of rates of metabolism in the population. Such individual variation is not impossible, however. Polymorphism in respect of debrisoquine hydroxylation in man results in large differences in its extent of oxidation between 'extensive metabolisers' and 'poor metabolisers' /162/. These differences extend to a number of other drug oxidations (such as guanoxan and phenacetin). A possible explanation is that 'extensive metabolisers' possess a form of cytochrome P-450 which metabolises these substrates, while 'poor metabolisers' are deficient in this enzyme.

Although information on the types and substrate specificities of human cytochrome P-450 is lacking, studies with the rat liver cytochrome P-450 enzyme system suggest that certain forms are more able to metabolise halogenated anaesthetics than are others. A single form of the enzyme appears to be responsible for enflurane metabolism, while methoxyflurane is metabolised by at least two forms /62/. Isoniazid appears to induce a form of the cytochrome which is particularly effective in metabolising the fluorinated ethers /32/. Biotransformation of halothane is carried out by a form of cytochrome P-450 inducible by phenobarbitone, but not by the 3-methylcholanthrene-inducible form /112, 163/.

Polymorphism with respect to a form of cytochrome P-450 with a high turnover rate for halothane could result in some individuals meta-

bolising halothane much more extensively than the general population. Studies of halothane metabolism in patients with liver dysfunction caused by halothane may determine whether this is the case. The opportunity for such studies is, however, rare.

In conclusion, the biotransformation of volatile inhalation anaesthetics plays a central role in their renal and hepatic toxicity. The potential hepatic toxicity of these drugs closely parallels their degree of metabolism in the order: chloroform > fluroxene > halothane > enflurane > isoflurane. Insufficient data exist to place methoxyflurane in this ranking. The renal toxicity of inhalation anaesthetics parallels their degree of metabolism to inorganic fluoride in the order: methoxyflurane > enflurane > isoflurane ≥ halothane.

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