Metabolism of the Volatile Anesthetics

Ellis N. Cohen, M.D.*

Until recently, all volatile anesthetics except trichloroethylene were considered to be inert substances, eliminated from the body without alteration. This concept is no longer acceptable, and evidence is now available to indicate that most inhalation anesthetics of hydrocarbon structure are metabolized in the body. Van Dyke and Chenoweth, Greene, Van Dyke, and Brown and Vandam have reviewed current progress in the field. The present effort is an attempt to fill the expanding gaps as new experimental data rapidly accumulate in pace with our widened interest in metabolism of the anesthetics.

Biodegradation of the volatile anesthetics clinically used has become an area of concern to both the anesthesiologist and the clinical pharmacologist. Recent investigations and developments have stimulated our interest in the implications of anesthetic metabolism. Among the significant questions to be answered are the possible toxic effects produced by anesthetic metabolites on vital organ systems such as the liver and kidney. Another serious problem is the development of sensitization responses to certain anesthetics in susceptible individuals. Although the etiologic factors responsible remain unidentified at present, it is conceivable that these result from the combination of free radicals with large protein fractions to form antigenic molecules. Of further concern is our knowledge that certain commonly administered drugs stimulate the metabolism of anesthetics, and that the anesthetics themselves may stimulate the metabolism of other inhalation anesthetics, as well as that of other drugs administered simultaneously. Finally, it has been suggested that induction of enzyme systems in the liver may occur in anesthetists and surgical personnel following their repeated exposure to the anesthetic-contaminated atmosphere of the operating room. This chronic exposure and enzyme induction results in increased rates of drug metabolism, with consequences as yet unknown.

Enzyme Induction and Inhibition

It has been recognized for some time that repeated administrations of a drug frequently lead to an altered response. Among the causative factors to be considered are the stimulation and the inhibition of drug-metabolizing enzymes. Such enzymes are known to be present in the endoplasmic reticulum of the liver and, to a lesser extent, in the brain and kidney. These enzymes do not appear to be substrate-specific, but may be categorized in terms of the chemical transformation produced, i.e., ether cleavage, dehalogenation, etc.

Early experiments with the volatile anesthetics indicate that pretreatment of an animal with any of a number of drugs stimulates the metabolism of anesthetics administered subsequently. Furthermore, both self-induction and cross-induction of metabolism between volatile anesthetics have been shown. Recently, a number of additional examples of induction phenomena have been demonstrated in vivo for the volatile anesthetic agents. It has also been shown that the volatile anesthetics may influence the biotransformation of other drugs administered concomitantly or subsequently. In-vitro studies suggest that these effects result from physical alteration in enzymic structure, alteration of the microsomal membrane, or a competition for "activated oxygen." Of practical importance, certain preservatives added to the liquid anesthetics, e.g., butylated hydroxytoluene and N-phenyl-1-naphthylamine, will themselves stimulate microsomal enzyme systems. Finally, of particular interest to the clinician is a recent study indicating that anesthetists as a group metabolize halothane more efficiently.

* Professor of Anesthesia, Stanford University School of Medicine, Stanford, California 94305.

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than pharmacists. The implication of this study is that repeated exposure of anesthetists to this or other anesthetic gases in the operating room markedly stimulates enzyme induction systems. Although enzyme induction in the above situation is slow and may involve weeks or months of exposure, induction of enzymes under other circumstances may begin within six hours and reach maximum levels within 24–48 hours.

Enzymic inhibition of anesthetic metabolism may also occur, and has been demonstrated in vivo following treatment with various antimetabolites such as SKF 525-A \(^7\) and disulfiram (tetraethylthiuram disulfide).\(^2\) Pretreatment with the latter actually serves to prevent the hepatotoxic effects of chloroform in rats, indicating that the damaging effects of this anesthetic may be due to "toxic metabolites." It has been suggested that the damaging effects following chloroform anesthesia may be related to binding of free radical intermediates to cellular constituents within the liver.\(^8\) Of further interest, chloroform hepatotoxicity cannot be produced in newborn animals, probably due to lack of development of drug-metabolizing enzyme systems.\(^4\)

It has also been shown that the rate of metabolism of an anesthetic may be influenced by the concentration being inhaled. When high concentrations of halothane, methoxyflurane, or fluoroene were inhaled, their own biotransformation was inhibited, while in trace concentrations these anesthetics are extensively metabolized.\(^9, 18, 67, 64\) High concentrations of anesthetic also reduce the metabolism of a variety of other drugs in a dose-dependent manner, suggesting saturation of the enzyme system by the anesthetic.\(^4, 13\)

Chronic Exposure to Subanesthetic Gas Concentrations

A number of recent studies indicate that trace amounts of anesthetic gases are free in operating rooms during and after the administration of anesthesia.\(^52, 54, 51\) Measurable concentrations of anesthetics have been demonstrated in the expired air of operating room personnel, as well as in the blood of anesthetists.\(^41, 70\) Although significant toxic effects resulting from these residual concentrations remain to be established, evidence has been presented for the induction of liver enzyme systems in anesthetists.\(^18\) It has been suggested that these concentrations may be sufficient to produce a sensitization response.\(^52\) Rats showed weight loss, hepatomegaly, and histologic changes in the liver following chronic exposure to low concentrations of halothane or methoxyflurane, but not diethyl ether.\(^56\) In rats exposed to lower concentrations (100 ppm) no changes were found when necropsies were done at six months.\(^52\) Of concern are preliminary reports suggesting a teratogenic effect, evidenced by the increased spontaneous abortion rate in pregnant nurses and doctors chronically exposed to residual anesthetic gases.\(^53, 49, 82\) The possible role of anesthetic metabolites in producing the above effects has not been evaluated.

Nonhalogenated Hydrocarbon Anesthetics

DIETHYL ETHER

Biodegradation of diethyl ether has been demonstrated following intraperitoneal injection of a \(^14\)C-labelled anesthetic into rats. Cleavage is presumed to occur at the ether linkage, resulting in formation of labelled carbon dioxide and the recovery of nonvolatile metabolites in the urine.\(^64\) In-vitro studies indicate that these ethers are cleaved under the catalysis of liver microsomal enzymes.\(^57\)

Recent low-temperature whole-body autoradiography studies in the mouse have indicated that 2.1 per cent of the intravenously administered radioactivity is localized in the liver as nonvolatile metabolites within two hours of administration. At that time, total-body radioactivity approximates 3.6 per cent of the administered dose.\(^25\) Separation and identification of in-vivo metabolites of diethyl ether were accomplished by thin-layer radiochromatography and mass spectrometry. These were shown to represent labelled fatty acids, cholesterol, mono-, di-, and triglycerides.\(^29\) From these findings it is reasonable to assume that diethyl ether is transformed to \(^14\)C-ace-tate, from which point it readily enters the common metabolic pool (fig. 1). Thus, from the metabolic point of view, diethyl ether may be considered to be a very safe anesthetic, degrading to nontoxic materials normally pres-
METABOLISM OF VOLATILE ANESTHETICS

CH₂CH₂CH₂CH₃ → CH₂CH₂CH₂OH → acetone

CH₂CH₂CH₂CH₃ → CH₂CO₂H → CO₂

Acetyl CoA

Fatty acids
Glycerides
Cholesterol
Carbohydrates, etc.

Fig. 1. Metabolism of diethyl ether.

ENT. This finding may account for its lack of toxicity following chronic exposure.56

ETHYLENE

Although this anesthetic may be produced normally by the liver mitochondria, it can be shown that 14C-labelled ethene administered to rats is converted to 14CO₂ and to nonvolatile labelled urinary products.66 Recent studies in mice using low-temperature, whole-body autoradiography also indicate the presence of high concentrations of nonvolatile metabolites in the liver. An ether extract of these metabolites may be separated into three components by thin-layer radiochromatography, but thus far these have not been identified.†

Cyclopropane

Unfortunately, only limited studies concerning the metabolism of this anesthetic are available. Data obtained in rats suggest that 14C-labelled cyclopropane is converted to 14CO₂. Due to numerous technical difficulties, quantitative data are not available.66

Halogenated Hydrocarbon Anesthetics

CHLOROFORM

Previous evidence has indicated that chloroform is metabolized both in vivo66 and in vitro.17 Biodegradation products were shown to include 14CO₂ and 36Cl-labelled nonvolatile urinary metabolites.44 Low-temperature whole-body autoradiographic studies in mice with 14C-chloroform have demonstrated that approximately 4 per cent of the radioactivity administered is present as nonvolatile metabolites in the liver and intestine within two hours.24 Additional nonvolatile metabolites are concentrated in the nasal mucous membranes and in the bronchi. The high fat/blood solubility coefficient assigned to this anesthetic (λ = 28) may be a significant factor in its metabolism, since the fat depots retain the volatile anesthetic within the body during the postanesthetic period, making it available to the processes of biodegradation over an extended period. 14CO₂ production continues at a steady rate for at least 12 hours after administration of the anesthetic.44

Thin-layer radiochromatography has established the presence of two nonvolatile chloroform metabolites in the liver. Although neither the identification nor the toxicity of these materials has been established, it has been suggested that the metabolism of chloroform may result in the formation of free radicals, which then combine with cellular constituents in the liver.99 Of interest is the action of the anti-metabolite, disulfiram, which acts to prevent both the metabolism and the hepatotoxicity of chloroform. Enzyme-inducing agents such as DDT and phenobarbital increase the hepatotoxic effects.22

FLUOROCENE

14C-labelled fluoroene has been shown to undergo biotransformation in both mouse and dog. The resulting metabolites were identified as trifluoroethanol glucuronide, trifluoroacetic acid, and 14CO₂.9 The latter was formed from the vinyl carbons only, since the trifluoromethyl group is not broken down to a significant degree (fig. 2). In the mouse, these metabolites account for 9.34 to 11.3 per cent of the injected dose, and the 14CO₂:to-urinary metabolite ratio was found to be 1:5. Pre-treatment of the mice with enzyme inducers, e.g., phenobarbital, 3-methylcholanthrene, and 3,4-benzpyrene, significantly increased the metabolism of fluoroene. The response, however,
is blocked by prior administration of actinomycin D. Hepatotoxic doses of chloroform also markedly decreased the metabolism of fluroxene, suggesting that the liver is the site of fluroxene metabolism. Pretreatment with diethyl ether or nitrous oxide had a slight stimulating effect on the biotransformation of fluroxene, but neither fluroxene nor methoxyflurane pretreatment produced significant changes.9

Studies in man with 14C-labelled fluroxene indicate that in two volunteers 12.1–15.4 per cent of the drug was excreted as nonvolatile urinary metabolites in 24 hours.11 Of interest were the similar rates of metabolism of fluroxene in these two individuals, despite a previously shown marked difference between their rates of halothane metabolism.18

HALOTHANE

Although halothane was originally introduced as one of the most stable of the hydrocarbon anesthetics, significant metabolism was soon demonstrated both in vitro and in vivo.64, 76, 77, 79, 80, 84 As expected, the carbon-to-fluorine bond was not easily broken, and relatively little 14CO2 formed (less than 1 per cent). The carbon-to-chlorine and the carbon-to-bromine bonds, however, were more readily broken by liver microsomal enzymes, which required the presence of reduced nicotinamide adenine dinucleotide phosphate (NADPH) and oxygen.85 By measuring the amount of halothane taken into the body during inhalation anesthesia, it was shown that 12–20 per cent of the halothane could be accounted for in terms of nonvolatile urinary metabolites recovered over a 13-day period.61 The major urinary metabolites have been suggested to be bromide ion61, 77 and trifluoroacetic acid.74, 79, 80

Fluoride present in the urine is organically bound, with less than ½ per cent as inorganic fluorine.61

Current studies have added to our information about the metabolism of halothane. Low-temperature whole-body autoradiographic experiments in mice have demonstrated the accumulation of large amounts of nonvolatile metabolites in the liver following the injection of 14C-halothane.56 These metabolites accumulate rapidly (9.2 per cent present in the body within two hours), but leave the liver only slowly. Residual concentrations are present for at least 12 days. These nonvolatile liver metabolites have been shown to be fluorine-containing molecules in which the ratio of fluorine to 14C remains unchanged from that of the parent 14C-halothane molecule. Autoradiography and thin-layer radiochromatography indicate the presence of many metabolites in a liver extract which contains only 65 per cent of the total radioactivity. The remainder of the radioactivity in the liver has thus far proven difficult to recover.

Induction of liver enzyme systems, leading to increased rates of halothane metabolism, follows the administration of inducing agents. Studies of halothane-anesthetized rats pretreated with phenobarbital showed a twofold increase in bromide excretion in urine collected over a five-day period when the rats were compared with a control group.22 In other studies, rats pretreated for 13 days with phenobarbital showed a fivefold increase in halothane metabolism, without evidence of liver damage.71 Enzyme induction in mice followed repeated intravenous administrations of 14C-labelled halothane and resulted in a 423 per cent increase in nonvolatile liver metabolites after five weekly administrations of halothane.56 Of considerable clinical interest has been the demonstration that anesthetists
as a group metabolized trace doses of $^{14}$C-halothane more efficiently than did a control group of pharmacists, suggesting stimulation of liver enzyme systems in the former following repeated exposure to halothane.\textsuperscript{19}

The elimination of large amounts of nonvolatile metabolites in the urine (10.6 to 23.2 per cent in five days) was demonstrated in nine anesthetists and pharmacists given $^{14}$C-labelled halothane, confirming earlier reports.\textsuperscript{61} Possible genetic and environmental influences on halothane metabolism have been studied recently in a small series of identical and fraternal twins. Intra-pair percentage differences between the amounts of nonvolatile urinary metabolites were less in the identical than in the fraternal twins, suggesting the greater influence of genetic rather than environmental factors.\textsuperscript{50}

Efforts have been made to identify the nonvolatile halothane metabolites found in the urine. Studies in the mouse and in the squirrel monkey indicate that the metabolites are many and are largely in the polypeptide and amino acid range, with only 33 per cent having molecular weights below 700. Small amounts of both trichloroacetic acid and a glucuronide were found, but no significant amount of nonvolatile urinary metabolites in this species presently remains unidentified.\textsuperscript{28} Recent studies in man utilizing thin-layer chromatography, gel filtration, and ion exchange have demonstrated only low-molecular-weight metabolites of halothane in the urine. The major peaks have been identified (gas chromatography and mass spectrometry) as the sodium salt of trichloroacetic acid and as trichloroacetyl ethanolamide.\textsuperscript{†} It is apparent that significant species differences in the metabolism of halothane exist among men, mice, and monkeys.

The isolation and identification of the nonvolatile halothane metabolites found in the liver have proven difficult. In the monkey and mouse livers, multiple chemical extraction procedures recover less than \% of the radioactivity present, suggesting that the metabolites are of high molecular weight. Further evidence indicates that the liver metabolites are intimately bound to large cell fragments present in cell membranes, microsomes, and mitochondria.\textsuperscript{29} Differential ultracentrifugation of the subcellular fractions in the liver shows the highest concentrations of radioactivity (count per mg) to be associated with the microsomes and the mitochondria. Similar findings have been demonstrated in man.\textsuperscript{†} It has been suggested that formation of the trichloroacetate ion represents a key step in the metabolism of halothane by the liver. This is a highly reactive material which readily combines with proteins, polypeptides, and amino acids.

Experimental data indicating that trichloroethanol may represent an intermediary step in the transformation of halothane to trichloroacetic acid have recently been presented. Labelled trichloroethanol given intravenously to man is recovered to a large extent in the urine as trichloroacetic acid.\textsuperscript{21} Trichloroethanol may also be formed as a metabolite through trichloroacetalddehyde\textsuperscript{2} (fig. 3).

Although the toxicity of halothane metabolites in man remains to be determined, there is evidence of hepatotoxicity in experimental animals following the administration of trichloroacetic acid,\textsuperscript{1,49} trichloroethanol,\textsuperscript{1,2,65} or

\textsuperscript{†}To be published.
trifluoroacetaldehyde hydrate. Trifluoroethanol has been shown to be the most toxic of these "presumed" halothane metabolites in vivo. It has been suggested that trifluoroacetaldehyde, or a compound derived from it, may be responsible for the toxic effect of trifluoroethanol. The mechanism of hepatic injury in experimental animals in relation to the various metabolites is poorly defined, but may include a decrease in glutathione-conjugating enzyme activity, inhibition of anaerobic glycolysis, binding of the halogen to a metal-protein combination, or a direct damaging effect of halothane metabolites on the mitochondrial membrane.

An increase in hepatic toxicity reactions has been reported to follow repeated exposure to halothane anesthesia. In a number of well-documented case reports, this appears to represent a sensitization response. Although the hapten responsible has not yet been discovered, it is unlikely to be represented by a small molecule like halothane. Large antigenic molecules, however, may result from the metabolism of halothane in which the reactive metabolites combine with protein. Evidence for the formation of such large molecules has been presented. An alternative explanation for this hepatotoxicity is that the metabolites themselves are directly toxic and accumulate to damaging concentrations within the liver following repeated anesthetic administrations. The toxicity of these materials in man is presumptive and remains to be established.

Additional studies have been carried out with 3H-labelled halothane prepared by activation analysis. These studies indicate the long-term presence of residual halothane metabolites in the body. It has also been shown that increased 3H blood levels follow repeated anesthetic administration at weekly intervals to the same individual.

**Methodology**

Metabolism of this anesthetic has been extensively studied. Early investigations using 14C- and 35Cl-labelled materials in rats indicated its degradation to 14CO2 and to non-volatile urinary 35Cl-labelled metabolites. Subsequent in-vitro studies demonstrated enzymatic biodegradation through the processes of dechlorination and O-demethylation. This may be shown to take place in rat liver slices, and requires the presence of both NADPH and oxygen. Induction of microsomal dechlorinating and ether-cleaving enzymes occurs in vivo following pretreatment of the animals with phenobarbital, methoxyflurane, or 3,4-methylenedioxymethamphetamine. The latter, unlike the former, was effective in vivo only. Of interest has been the report that sodium pentobarbital increases the deposition of fluoride in bones following methoxyflurane anesthesia, presumably on the basis of increased methoxyflurane metabolism. Ethanol, on the other hand, depresses this response in the mouse.

Recent studies in man, using tracer doses of 14C-labelled methoxyflurane, confirm the earlier animal studies. Biodegradation was shown to begin immediately after exposure and continue for 9-12 days when storage depots of intact drug approach depletion. Seven to 21 per cent of the methoxyflurane administered underwent ether cleavage, and in one subject an additional 40 per cent was dechlorinated. In these studies, the metabolites of methoxyflurane were identified as dichloroacetic acid, methoxydifluoroacetic acid, and free fluoride ion. Additional reports confirm the presence of increased amounts of inorganic fluoride in the sera of patients undergoing methoxyflurane anesthesia. Increased levels of inorganic fluoride in rats have been shown to be associated with polyuric renal insufficiency. The presence of calcium oxalate crystal deposits in the proximal tubules of the kidney has also been reported. These appear to contribute to the severity of the renal failure, although not to account for it completely. The interrelationship of these metabolites and proposed pathways for the biodegradation of methoxyflurane have been presented recently (fig. 4). Since it has been suggested that nephrotoxicity may relate to the amount of anesthetic metabolized (measured as increased fluoride levels), the clinical implications of the metabolism are apparent. Additional reports of renal toxicity are beginning to accumulate.

**Ethane and Forane**

These new inhalation anesthetics are presently under clinical investigation. Preliminary data on their metabolism indicates that they undergo limited biodegradation. The metabolism of Ethane in mice was demonstrated to
be 2.7 per cent that of methoxyflurane on the basis of fluorine deposition in bone.\textsuperscript{33} Studies with Ethane in man indicate that only 2.4 per cent of the administered Ethane is recovered as urinary fluorine, including both organic and inorganic forms.\textsuperscript{33} Liver-perfusion studies in the pig using Forane anesthesia thus far have been unable to demonstrate any metabolites in the portal drainage (gas-liquid chromatography).\textsuperscript{32} Confirmation of this lack of metabolism in man will offer a new concept in halogenated hydrocarbons available for anesthetic use.

\textbf{TRICHLOROETHYLENE}

This anesthetic agent is infrequently used and is of interest only from a historical point of view. Its metabolism was established as early as 1933.\textsuperscript{14} Butler postulated that the first step in the metabolism of trichloroethylene was conversion to chloral hydrate.\textsuperscript{16} Trichloroacetic acid has been demonstrated in human blood, and both trichloroacetic acid and trichloroethanol glucuronide have been found in the urine.\textsuperscript{19, 75}

\textbf{Inorganic Anesthetics}

\textbf{NITROUS OXIDE}

At the present time there is no evidence to indicate that metabolism of this anesthetic occurs. However, nitrous oxide is not biologically inert, and effects on the hematopoietic system \textsuperscript{31} and the reticuloendothelial system \textsuperscript{32} have been described. Tertogenetic effects have also been reported.\textsuperscript{33, 74} Furthermore, enzyme induction has been shown to be produced by nitrous oxide, which in turn at least suggests that it should be metabolized.\textsuperscript{30} It is likely, therefore, that the metabolism of nitrous oxide will be demonstrated as adequate technology becomes available. Stable isotopes of nitrous oxide have been prepared, but a satisfactory radioactive form of nitrous oxide does not exist.

\textbf{Conclusion}

Although evidence as to the biodegradation of most volatile hydrocarbon anesthetic agents is available, more information must be forthcoming before the full implications can be
properly understood. Certainly, anesthetic metabolism plays little role in the clinical conduct of anesthesia, and the anesthetist is almost totally unaware of its occurrence as fresh gases continue to be supplied to his patient in large amounts. On the other hand, possible toxic effects of metabolism on major organ systems, such as the kidney and liver, are beginning to be recognized.

The problem is a difficult one. While the incidence of such untoward reactions to anesthetics is fortunately very small, the consequences to the individual are serious, and sometimes fatal. From the investigator’s point of view there are but few cases to study, and even these show wide variation in responses. Furthermore, species differences between man and animal limit the use of many animal model systems. Technical difficulties related to the handling of trace metabolites in microgram quantities, intimately bound to complex molecules, abound. Nonetheless, the problem is important and cannot be ignored. The challenge is great, and answers must be provided.

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