Absorption, Biotransformation, and Storage of Halothane

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Current knowledge of the quantitative aspects of biotransformation of halothane and the fate of its metabolites are reviewed. Absorbed quantities of the inhalation anesthetic average 12.7 and 18 g during 1 and 2 hr, respectively, of anesthesia. Reported fractions of halothane recovered as urinary metabolites range from 10 to 25%. An analysis of reports of bromide ion accumulation in plasma during and following anesthesia suggests that metabolism of halothane continues for 20–40 hr after exposure and that 22–24% of absorbed halothane is metabolized following 8 hr of anesthesia. Half-times for excretion of trifluoroacetic acid (TFA), a principal urinary metabolite of halothane, tend to confirm that biotransformation proceeds for 2 to 3 days following exposure. Other urinary metabolites which occur in small amounts include a dehydrofluorinated metabolite of halothane conjugated with L-cysteine and N-trifluoroacetyl-n-ethanolamine, both of which are evidence of the occurrence of reactive intermediates during the metabolism of halothane. Support for free radical formation has come from in vivo and in vitro demonstrations of stimulation of lipoperoxidation of polyenoic fatty acids by halothane. Irreversible binding of halothane metabolites to microsomal proteins and phospholipids has been shown to depend on the microsomal P-450 cytochrome system. Irreversible binding is increased by microsomal enzyme induction and by anaerobic conditions. Hypoxia increases irreversible binding to phospholipids, augments the release of inorganic fluoride and is followed by centrilobular hepatic necrosis. It is concluded that one-fourth to one-half of halothane undergoes biotransformation in man. One fraction is excreted as trifluoroacetic acid, chloride and bromide. A second fraction is irreversibly bound to hepatic proteins and lipids. Under anaerobic conditions fluoride is released, binding to phospholipids is increased, and hepatic necrosis may occur.

The purpose of this communication is to review current knowledge of the quantitative aspects of biotransformation of halothane and the fate of its metabolites.

Absorbed Dose

In order to establish anesthesia as soon as possible, inhalation anesthesia is commonly initiated by using overpressure, i.e., an inspired concentration higher than the minimum alveolar concentration (MAC) (1) required to maintain anesthesia. Progressive reduction of the inspired concentration is employed to maintain a constant alveolar (and arterial blood) concentration. The whole body cumulative uptake depends on the solubility of the anesthetic in all the tissues, the volumes of the tissues and the rates of blood flow to the various tissues. Lowe (2) has shown, however, that the cumulative uptake (Qan) required to achieve and maintain a constant plane of anesthesia can be approximated by the expression

\[ Q_{an} = 2 \times 1.3 \text{MAC} \times \lambda_b Q t^{\frac{1}{2}} \]

where \( \lambda_b \) is the blood/gas partition coefficient of the anesthetic, Q is the cardiac output, and t is the duration of the exposure.

Reported cumulative uptakes (3–7) for inhalation anesthetics average about 18 g during 2 hr of anesthesia (Table 1) or, by the square-root-of-time rule given above to estimate, 12.7 g during 1 hr of anesthesia. These quantities of drug absorbed during anesthesia set the anesthetics apart from other drugs and atmospheric pollutants, and prompt special consideration with respect to biotransformation and potential toxicity.

Factors Determining Extent of Biotransformation

The volatile anesthetics are metabolized to a greater or lesser extent, depending on their solubil-
flurane, that chemical stability must play an important role in limiting biotransformation, since these drugs are metabolized much less than is predicted by the model. Predictions for fluroxene, halothane, and methoxyflurane, on the other hand, appear to be substantiated by the experimental data given in Table 3.

Excretion

The production of metabolites of halothane by man have been measured mainly in urine and blood: sweat and feces have been shown to account for less than one percent of the dose (10). Stier et al. (12) demonstrated increased bromide excretion in urine. Rehdor (6) and associates accounted for 12% excreted as trifluoroacetic acid (TFA) by two patients. Cascorbi (10) confirmed that TFA was the principal fluorine-containing urinary metabolite of

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**Table 1. Cumulative uptakes for inhalation anesthetics.**

| Anesthetic | Total uptake | Exposure (avg., min. Reference |
|------------|--------------|-----------------------------|
|            | n Avg. Range |                             |
| Isoflurane | 9  18.1 10-24 | 130 (3)                     |
| Enflurane  | 7  18.4 15-32 | 115 (4)                     |
| Fluroxene  | 9  32.0 18-45 | 142 (5)                     |
| Halothane  | 2  9.2  7.3, 11.1 | 75 (6)                     |
| Methoxyflurane | 12  18.1 7.6-31 | 138 (7)                     |

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**Table 2. Simulated metabolism by a nonlinear model.**

| Anesthetic | Anesthetic metabolized, % | Anesthetic metabolized, mmole* |
|------------|---------------------------|--------------------------------|
|            | 1 hr 4 hr 8 hr           | 1 hr 4 hr 8 hr                 |
| Fluroxene  | 1.4 34 12 13 14 13 30 48 |                                |
| Diethyl ether | 13. 50 15 12 13 73 110 140 |                                |
| Isoluroxene | 1.4 68 16 18 20 12 30 50  |                                |
| Enflurane  | 1.9 70 17 18 20 11 26 42  |                                |
| Fluroxene  | 2.4 155 24 26 30 14 36 59  |                                |
| Methoxyflurane | 11. 670 51 52 54 27 64 100 |                                |

*Data of Feingold and Holaday (7).
*For various durations of anesthesia.
*Blood-gas partition coefficient.
*Fat-gas partition coefficient.

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**Table 3. Fate of inhalation anesthetics in man.**

| Anesthetic | n | Exhaled | Metabolites | Total | Not recovered, % | Reference |
|------------|---|---------|-------------|-------|------------------|----------|
| Isoflurane | 9 | 95      | 0.17        | 95    | 5                | (3)      |
| Enflurane  | 6 | 83      | 2.4         | 85    | 15               | (4)      |
| Fluroxene  | 9 | 58      | 10          | 68    | 32               | (5)      |
| Halothane  | 1 | 46*     | 25          | 71    | 29               | (10)     |
| Methoxyflurane | 12 | 19      | 44          | 63    | 37               | (7)      |

*Assumes 80% exhaled in 5 hr.
halothane and amounted to 10.6 to 24.8% (avg. 17%) of an intravenously infused dose in unanesthetized volunteers, and about 30% less following anesthesia. Anesthesia also decreased the half-time of urinary metabolites in one subject from 55 to 45 hr. In their most carefully controlled experiment, 62% of the injected dose was recovered, 36.8% in exhaled air, 24.8% in urine, 0.336% in sweat and feces. Recovery in exhaled air was reported for 5 hr only; on assuming that 80% of the amount to be exhaled was exhaled in 5 hr, recovery can be corrected to 71%. In other experiments 58 to 74% was recovered. This is consistent with our findings in balance studies of other anesthetics (13) (Table 3). Exhaled air was sampled for periods of 5–10 days and urine was collected for up to 14 days. Essentially the same methods of collection were used in all studies. These data suggest that the larger the fraction recovered as urinary metabolites, the larger the fraction that cannot be accounted for as exhaled unaltered drug and urinary metabolites.

Accumulation of Metabolites in Body Fluids

An alternative way of accounting for extent of biotransformation is to measure the accumulation of metabolites in body fluids. This requires measurement of the metabolite in plasma, and knowledge of the binding characteristics, the apparent volume of distribution and the time constants for distribution and excretion of the metabolite. Bromide ion release from halothane presents a reasonable index for following halothane degradation. Bromide is a principal metabolite, is distributed mainly in the chloride space, and is excreted very slowly by the kidney ($t_{1/2} = 12–22$ days).

Plasma bromide concentrations during and following anesthesia have been studied (14–17). Tinker and Van Dyke (14) found that peak plasma bromide concentration rose linearly in patients at a rate of 26 mg/l. per MAC hour. The total dose of an anesthetic is the sum of the amounts taken up by the rapidly perfused visceral organs, muscle, slowly perfused aqueous tissues, fat, and the amount metabolized (13). The viscera achieve equilibrium in 20 min and account for 72% of cumulative total body uptake at that time. Muscle and other lean body tissues account for 16% of the dose at 20 min and continue to absorb at a slow, decrementing rate for several hours, while fat continues to absorb drug at an almost constant rate for as long as anesthesia lasts (2). The anesthetics are released in the same order. Accordingly, Tinker’s observation of a linear rate of rise of bromide in plasma suggests that prolonged release of halothane from the slowly exchanging compartments, rather than the total absorbed dose, determines the rate and extent of biotransformation.

Relatively little bromide is released during anesthesia (15, 16) (Table 4). The major portion of plasma bromide accrues during 20–40 hr following the end of anesthesia (15–17). This approximates the half-time for depletion of the slowest exchanging storage compartment.

Knowledge of the half-time of urinary trifluoroacetic acid also provides an opportunity for estimating when biotransformation occurs, during exposure when blood concentrations are high, or during recovery when slow concentrations are maintained by diffusion from slowly perfused lipid storage areas. Holaday and Cunnah (18) found that the half-time for urinary excretion of TFA by normal volunteers is 16 hr. The half-times for excretion of TFA by patients calculated from the data of Rehder et al. (6) was 58 hr and from the data of Cascorbi et al. (10) on volunteers, the half-times cover a range from 39 to 61 hr. If it is assumed that diffusion of TFA out of cells occurs within a few hours, the difference between these half-times suggests that the predominant portion of biotransformation occurs after anesthesia during the first 2 or 3 days following anesthesia.

### Biotransformation and Toxicity

Jaundice and hepatic necrosis are rare toxic reactions which follow halothane anesthesia. The mechanism responsible for this has eluded any proven explanation but consistently has been linked to biotransformation, and covalent binding to vital subcellular macromolecules has been implicated.

| Subjects | Exposure | Change (Br⁻) | Dose metabolized, %<sup>a</sup> | Reference |
|----------|----------|--------------|-------------------------------|-----------|
| 8 patients | 1.5%, 20 min | 2.3/30 min | 0.68 | (15) |
| | | 22.85/20–40 hr | 11.9 | |
| 7 volunteers | 1.0%, 6.6 hr | 200/48 hr | 21.8 | (17) |
| 7 volunteers | 1.0%, 8.9 hr | 12.8/8 hr | 0.55 | (16) |
| | | 216/40 hr | 24.4 | |

*aAbsorbed dose calculated after Lowe (I); bromide space taken to be 0.275 l./kg body weight.

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lites, human (19). TFA and the concurrently formed metabolites, \( \text{Br}^- \) and \( \text{Cl}^- \) are essentially nontoxic, and TFA has been shown not to bind (19, 20).

Cohen et al. (21) have demonstrated the occurrence of organic metabolites other than TFA in human urine. They collected urine from heart-donors for 6 hr following infusion of \( ^{14}\text{C}- \text{halothane} \). They were able to identify three large radioactive peaks and several smaller peaks by anion-exchange column chromatograph. The largest and most acidic peak dominating later urines proved to be trifluoroacetic acid. A less acid fraction which appeared only in early samples was identified as a thio ether of \( N\)-acetylcysteine and a dehydrofluorinated metabolite of halothane; \( N\)-acetyl-S-(2-bromo-2-chloro-1,1-difluoroethyl)-L-cysteine. A third, neutral, water-soluble fraction appearing in constant, low concentration was \( N\)-trifluoroacetyl-N-ethanolamine. Trace quantities of the two conjugated metabolites were also identified in the urines of five normal volunteers given tracer dose of \( ^{14}\text{C}- \text{halothane} \). The two conjugated compounds imply the occurrence of reactive intermediates in the metabolism of halothane.

Brown and his associates (22, 23) have explored the possibility that free-radical intermediate metabolites of halothane stimulate lipoperoxidation of polyenoic fatty acids. These are components of the phospholipids which contribute to the structure of the endoplasmic reticulum. Their disruption could cause interference with vital functions of the microsomal enzyme system. In phenobarbital-pretreated rats, but not in non-induced animals, halothane increased diene conjugate formation, as did chloroform but not fluoroene or diethyl ether (22). Chloroform depleted glutathione and caused centrilobular liver necrosis, whereas halothane did not (23). These observations provide some support for Green's conclusion from a retrospective clinical study that induced patients are not at more risk from halothane anesthesia than non-induced patients.

Van Dyke and his associates have elucidated the locations and determinants of irreversible binding of halothane metabolites in rat liver (19, 20, 24–27). Binding occurs in the microsomes (19), is inhibited by carbon monoxide (19, 24) and SKF-525A (25), requires NADPH (24), is increased by pretreatment with phenobarbital (19) and polychlorobiphenyls (25), but not by methylcholanthrene (19, 25), and is greatly accelerated by anaerobic conditions (24, 26, 27). Under aerobic conditions in vivo, most binding is to microsomal proteins and phospholipids (24, 27). In animals breathing halothane in 7% oxygen, total binding was doubled and the lipid/protein binding ratio increased more than fourfold. Fluoride release, normally negligible, increased twentyfold in surviving animals and, in animals which died during exposure, to 70 mmole/l. in plasma, a 100-fold increase (27).

\( ^{14}\text{C} \) and \( ^{36}\text{Cl} \)-labeled halothane bound equally to phospholipids under anoxic conditions (26), suggesting that the chlorine atom remained attached to the halothane moiety bound to the lipids. It is possible that this moiety is the same dehydrofluorinated metabolite that Cohen (21) found conjugated to L-cysteine in human urine.

Wood et al. (26) confirmed Brown's reports (22, 23) of lipoperoxidation of polyenoic acids in oxygenated \textit{in vitro} incubations of induced microsomes with halothane. In anaerobic incubations, diene conjugate formation progressed as under aerobic conditions, but peroxidation was inhibited, and binding of a halothane metabolite to phospholipids was increased greatly (26). This suggests that oxygen binds more readily to diene conjugates than a halothane intermediate. Oxygen may also oxidize the halothane intermediate to TFA before it can bind.

## An Animal Model of Halothane Hepatotoxicity

Most exposures of experimental animals to halothane have been benign. However, even when exposures have been conducted under severe hypoxia or following multiple prolonged exposures of phenobarbital-pretreated animals and toxicity has been evident (28), the centrilobular necrosis which pathologists associate with clinical halothane hepatitis (29) has not been mimicked.

Recently Sipes and Brown (30) have proposed an animal model of halothane hepatic necrosis. Centrilobular hepatic necrosis was demonstrable in Aroclor 1254 [a mixture of polychlorinated biphenyls (PCB)]-pretreated rats 24 hr after exposure to 1% halothane in oxygen for 2 hr. \textit{In vitro} binding of \( ^{14}\text{C} \) from labeled halothane to microsomal proteins and lipids was also shown to be enhanced by PCB pretreatment in a pattern similar to that obtained by incubating microsomes with halothane in an atmosphere of nitrogen. Since PCBs are known to cause neoplastic changes in livers of mice (31), and halothane reduces hepatic blood flow (32), the effect may result from a combination of enhanced and altered microsomal activity, stagnant hypoxia, and the presence of other reactive intermediate metabolites.
Summary

The foregoing observations suggest that patients metabolize one-fourth to one-half of absorbed halothane. One fraction is excreted in the urine as trifluoroacetic acid, bromide, and chloride. Under normal conditions, a second fraction undergoes irreversible binding to microsomal proteins and lipids without interfering with cell functions. Under anaerobic conditions, a third pathway of biotransformation is activated, resulting in release of fluoride and increased binding to phospholipids. Hepatotoxicity may be associated with this third reaction.

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