

A Noninvasive In Vivo Method of Assessing the Kinetics of Halothane Metabolism in Humans

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The authors describe a noninvasive method of estimating the kinetic constants that characterize metabolism of inhaled anesthetics in humans. Ten healthy male volunteers breathed subanesthetic concentrations of halothane and isoflurane in a fixed inspired ratio of 20:1. Isoflurane served as a marker that identified changes in uptake in nonmetabolizing depots. Each study progressed through nine 30-min levels (numbered 0-8). At each level, inspired concentrations of both halothane and isoflurane were doubled, and alveolar concentrations and uptakes were determined. Clearance (uptake/alveolar concentration) of isoflurane remained constant over a range of concentrations of 0.00006 to 0.008%. In contrast, clearance of halothane decreased as the alveolar concentration increased from 0.0007 to 0.13%. On this basis, the authors assumed that the clearance of halothane was a combination of linear clearance to depots and saturable metabolism, the former proportional to the clearance of isoflurane, and the latter attributable to a Michaelis-Menten process. Applying such a model to halothane, they estimated the mean (\pm SE) V_{\max} (the composite maximum rate of metabolism) to be $0.79 \pm 0.09 \text{ ml} \cdot \text{min}^{-1} \cdot \text{individual}^{-1}$, and the K_m (the composite concentration at which half-saturation of enzymes occurs) to be $0.029 \pm 0.003\%$. This model provides a significantly better data fit than that provided by two simpler submodels, one of which assumes that all clearance is linear, and the other of which allows a part of clearance to be saturable but ignores the isoflurane marker data.

The value of 0.029% for K_m indicates that a wide range of clinical anesthetic concentrations will produce similar rates of metabolism; that metabolism will proceed at near maximum rates during the first several minutes of recovery; and that most metabolism probably occurs after, rather than during, anesthesia. (Key words: Anesthetics, volatile; halothane; isoflurane. Metabolism. Pharmacokinetics: kinetics; models; solubility. Recovery.)

IN THE EARLY 1960s, Paul and Rubinstein¹ and Van Dyke and co-workers^{2,3} demonstrated that inhaled anesthetics were metabolized. This discovery became important when some products of biodegradation of anesthetics were found to lead to organ toxicity.⁴ For example, renal failure was associated with methoxyflurane⁵; and hepatic injury with halothane.⁶ The metabolism of

an anesthetic may also be important to its pharmacokinetics. As much as 50% of the methoxyflurane that is taken up is excreted as metabolites.⁵

Most *in vivo* studies in humans or animals have measured metabolism by determining the amount of metabolites produced and/or the difference between uptake and retrieval of an agent.^{5,7,8} Using the latter technique, we recently found the metabolism of halothane to depend on concentration: at higher inspired concentrations, a lower percentage of the halothane taken up was metabolized.⁸

Two *in vivo* studies approached anesthetic metabolism from a kinetic viewpoint. Sawyer *et al.*⁹ showed an inverse relationship between the concentration of halothane and its fractional extraction in the liver of miniature swine. At very low partial pressures of halothane, the liver extracted 60 to 80% of the halothane brought to it. However, as the concentration increased, this percentage decreased; and at full anesthetizing partial pressures, fractional extraction fell toward zero. Similarly, Anderson and co-workers¹⁰ demonstrated that rats absorbed proportionately more halothane at low concentrations than at high ones. These results may be due to substrate (anesthetic) saturation of the enzymes responsible for metabolism, suggesting that this process follows Michaelis-Menten characteristics. The present study demonstrates that the kinetics of halothane metabolism can be defined quantitatively using noninvasive methods.

Methods

The study was approved by the Committee on Human Research at the University of California, San Francisco, and each subject gave informed consent. We studied ten healthy, lean, 21- to 31-year-old male volunteers, who reported no history of cigarette smoking, ethanol intake in excess of 22 ml/day, or intake of illicit or prescribed drugs except for the occasional use of marijuana. During the study, all subjects were seated and breathed predetermined inspired concentrations of gases from calibrated cylinders via a reservoir system attached to a low-resistance, nonbreathing valve used in conjunction with an air-tight mouthpiece and nose-clip. Expired gases were delivered through a mixing chamber to a Collins spirometer. Each study consisted of nine successive 30-min levels (numbered 0-8) in which subjects were exposed to progressively higher concentrations of anesthetics.

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Each level began with a 4-min loading period, after which the inspired concentration was reduced to one-fourth the loading concentration for 26 min. At each level, all concentrations were doubled over the previous level.

At the 4th, 20th, 25th, and 30th min of every level, we measured end-tidal (ET), mixed-expired (ME), and inspired (IN) concentrations for both gases, as well as expired minute ventilation (\dot{V}). From these values we calculated gas uptake [$U = (IN - ME) \cdot \dot{V}$] and clearance ($Cl = U/C$), where C is the alveolar (ET) concentration.

All gas samples were drawn with 50-ml airtight syringes and analyzed within minutes. A Tracor Model 220 gas chromatograph was used for separation and detection of halothane and isoflurane. A 10% S.F. 96 on Chromasorb® WHP, 6/80-mesh, column (1/8 in by 360 cm) was maintained at 82° C. A nitrogen carrier flow rate of 80 ml/min delivered the sample through the column to a flame ionization detector at 200° C, which was supplied by 40 ml/min of hydrogen and 283 ml/min of air. Retention times were 47 s for isoflurane and 72 s for halothane.

The initial 4-min period of equilibration, during which concentration was four times higher than in the ensuing 26 min, helped to saturate lean, slowly equilibrating compartments (*e.g.*, muscle). Since they have short time constants, highly perfused tissues (brain, heart, kidney, liver, splanchnic bed) became saturated in any case. Tissues with long time constants, primarily fat, probably remained essentially unsaturated at any level. During the final 10 min of each level (at 20, 25, and 30 min), anesthetic uptake presumably occurred because of metabolism or uptake in tissues with long time constants (for muscle, about 100 min; for fat, about 1,500 min) and, thus, should have been constant.

Each premixed cylinder contained subanesthetic concentrations of halothane and isoflurane in a fixed ratio of 20:1 in a mixture of 30% oxygen and 70% nitrogen. With each doubling of the concentration of halothane, the concentration of isoflurane (tracer) also was doubled. During the final 26 min of each level, the inspired concentrations of halothane and isoflurane (\pm SD) ranged from 0.00137 (0.00005) and 0.000072 (0.000005) at level 1, to 0.196 (0.006) and 0.00962 (0.00024)% at level 8. Isoflurane served as a marker that identified changes in ventilation and tissue perfusion that affected uptake by tissue depots. We assumed that isoflurane would be distributed throughout the body in a fashion comparable to halothane, but that it would not undergo significant metabolism. Furthermore, at one-twentieth the inspired halothane concentration, we assumed isoflurane would not affect the metabolism or distribution of halothane.

Four model equations (see next section) were tested using the general linear test (F test) to ascertain which

best described the kinetics of halothane metabolism. For each individual, least-squares regression analyses were performed for each model equation using the BMDP statistical package (programs P1R and P3R versions 2).¹¹ Data from level 0 (initial loading) were not used. Individual points from samples at 20, 25, and 30 min were used in all model fitting. Thus, 24 sets of data were used for each individual to determine the fit to each model equation.

MODEL EQUATIONS

If V_{\max} is the maximum rate of metabolism and K_m is the concentration at which half-saturation occurs (and at which one-half V_{\max} is attained), then halothane clearance due to metabolism (Cl_m) at any concentration (C_H) can be modelled as:

$$Cl_m = V_{\max}/(K_m + C_H). \quad (1)$$

Also,

$$Cl_H = Cl_d + Cl_m. \quad (2)$$

where Cl_H is total halothane clearance at the lung, and Cl_d is the clearance to depots where no metabolism occurs. Then,

$$Cl_H = Cl_d + V_{\max}/(K_m + C_H). \quad (3)$$

Two approaches may be used to assess the relative contributions of metabolism and inert depot storage to Cl_H . The first approach is simply to assume that Cl_d is constant. The second is to determine relative changes in depot clearance using a second gas, one that is not metabolized to a significant degree, but whose clearance to inert depots (Cl_f) is linearly related to the Cl_d of halothane ($Cl_d = \alpha Cl_f + \beta$). Thus, we can write the general equation:

$$Cl_H = \alpha Cl_f + \beta + V_{\max}/(K_m + C_H) \quad (4)$$

where α and β are constants.

When Cl_H , Cl_f , and C_H are determined experimentally at numerous C_H values, a nonlinear regression analysis may be used to estimate V_{\max} , K_m , α , and β . Three simpler submodels were also evaluated.

Submodel equation 1 was:

$$Cl_H = \alpha Cl_f + V_{\max}/(K_m + C_H),$$

where $\beta \rightarrow 0$, which assumes that all clearance of halothane that is not saturable is proportional to Cl_f .

Submodel equation 2, in which the isoflurane tracer is ignored, was:

$$Cl_H = \beta + V_{\max}/(K_m + C_H);$$

that is, $\alpha \rightarrow 0$, which assumes that all clearance of halothane that is not saturable is independent of Cl_f .

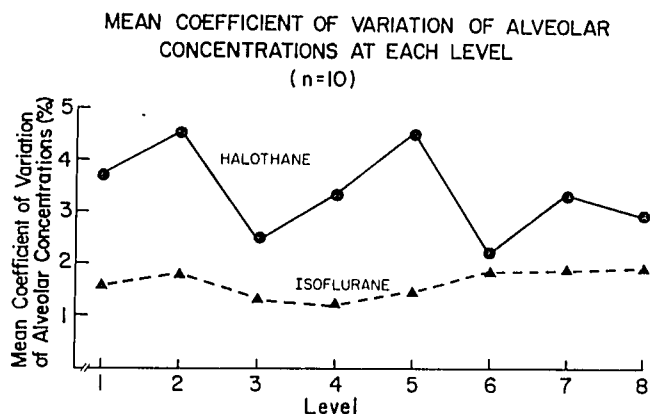


FIG. 1. Alveolar concentrations of both isoflurane and halothane were stable during the final 10 min of each level (when the sampling was done). The mean coefficient of variation is found by computing the mean and SD of the alveolar concentrations at 20, 25, and 30 min at each level ($n = 3$). Within each level, the mean of this ratio (SD/mean) over all volunteers ($n = 10$) $\times 100$ equals the coefficient of variation.

Submodel equation 3, in which no saturable process is assumed, was:

$$Cl_H = \beta + \alpha Cl_I,$$

where $V_{\max}/(K_m + C_H) \rightarrow 0$.

Isoflurane was utilized as the tracer gas. One of two assumptions must hold if it is to be considered a legitimate marker of nonmetabolic clearance. Either its metabolism must be a trivial part of its total clearance under the experimental condition used; or, if not, its metabolism must not be saturable throughout the concentration range used.

Results

As described in the methods section, the inspired concentration of isoflurane tracer was always one-twentieth the inspired concentration of halothane. At each level, the inspired concentration of each agent was fourfold higher during the first 4 min than during the final 26 min; and the inspired concentrations were doubled with each successive level.

At each level, the alveolar concentrations at 20, 25, and 30 min showed minimal variation (fig. 1). Alveolar concentrations (\pm SD) of halothane and isoflurane ranged from 0.00070 (0.00003) and 0.000059 (0.000005)% at level 1, to 0.135 (0.008) and 0.00786 (0.00031)% at level 8.

Isoflurane clearance is constant throughout those concentrations used; in contrast, halothane clearance is saturable (fig. 2). As judged by an analysis of residual sums of squares (table 1), the general equation does not provide a statistically better fit for our data than does the simpler

submodel equation 1 [$Cl_H = Cl_I + V_{\max}/(K_m + C_H)$]. However, submodel equation 1 does provide a significantly better fit than either submodel equation 2 (in which the isoflurane tracer data are ignored) or submodel equation 3 (the linear clearance submodel). A representative fit using submodel equation 1 is presented (fig. 3). The mean and standard error (for all ten volunteers) of the estimates of V_{\max} and K_m for halothane, as determined by submodel equation 1, are:

$$V_{\max} = 0.79 \pm 0.09 \text{ ml} \cdot \text{min}^{-1} \cdot \text{individual}^{-1},$$

and

$$K_m = 0.029 \pm 0.003\%.$$

Discussion

Our results agree with those from *in vivo* studies in animals. Using gas uptake studies from a closed chamber, Anderson *et al.*¹⁰ estimated the V_{\max} of halothane in the rat to be $0.014 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (compared with our V_{\max} of $0.012 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, and the K_m to be 0.0072%. Analysis of the work by Sawyer *et al.*⁹ in miniature swine produces remarkably similar results (V_{\max} of $0.015 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ and a K_m of 0.04%).

Four mechanisms might account for the fact that our results documented a concentration dependence for halothane clearance: 1) perfusion-limited metabolism in which the delivery of substrate (halothane) to the metabolic site is the rate-limiting step; 2) carrier saturation in which substrate supply exceeds the capacity of some

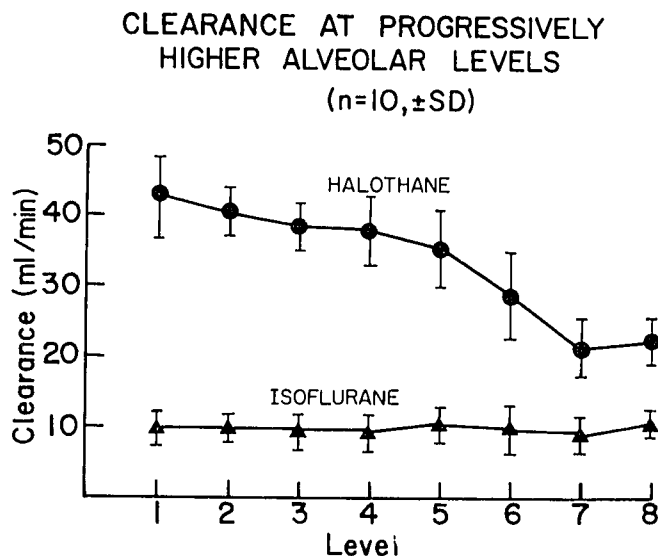


FIG. 2. The mean clearances of isoflurane remained constant regardless of concentration, while halothane clearances decreased, particularly over levels 5-7, and then stabilized (compare the results at levels 7 and 8).

carrier mechanism responsible for transport to the metabolic site; 3) substrate inhibition in which progressively higher concentrations of halothane inhibit their own metabolism; and 4) enzyme saturation in which substrate supply exceeds the capacity of the metabolizing enzymes.

We found that total clearance of halothane stabilizes at concentrations substantially higher than the apparent K_m of halothane (levels 7 and 8, fig. 2). A perfusion-limited model of metabolism would not account for this phenomenon.

Carrier saturation could account for our results. However, halothane is a relatively small, lipid-soluble molecule that should pass membrane barriers easily without the aid of a carrier.

Substrate inhibition is more difficult to rule out as an explanation. Our data also would be consistent with an absolute decrease in halothane metabolism when the higher alveolar levels of halothane were attained.

Adler *et al.*¹² demonstrated with isolated enzyme systems that no substrate inhibition occurs with methoxyflurane. No comparable studies on halothane have been reported. Anderson and co-workers¹⁰ cite preliminary results showing excellent correlation between the actual rate of bromide production and the predicted rate derived from Michaelis-Menten kinetic constants that were determined by studies of halothane uptake in rats. Similarly, we believe our results can best be explained using an enzyme saturation model.

Michaelis and Menten originally described a model for predicting the rate of reaction at steady state for a single-enzyme system.¹³ Our use of a comparable model and terminology to describe the *in vivo* metabolism of halothane bears special comment.

In using such a model, we have not assumed that a single-enzyme system is responsible for the rate-limiting

VOLUNTEER #1
OBSERVED (●) AND PREDICTED (line)
CLEARANCES AT PROGRESSIVELY HIGHER
ALVEOLAR LEVELS

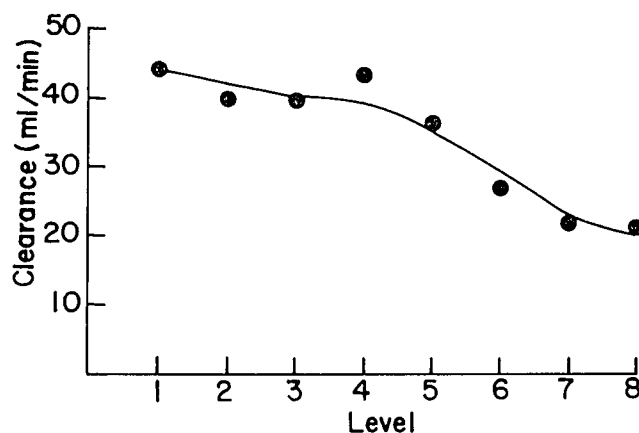


FIG. 3. The observed and predicted values of halothane clearance (submodel 1) for a representative volunteer are shown.

step of all halothane metabolism; nor have we assumed, should multiple systems be involved, that they have identical kinetic parameters. The excellent fit of our data to a Michaelis-Menten-like model does suggest that the composite parameters we determined describe the overall rate of reaction for the bulk of halothane metabolism, and that the underlying reason for this fit is saturation of the involved enzyme system(s).

The variability of α , V_{max} and K_m in our results is not surprising, and is due to biologic differences and our experimental methods. Both genetic and environmental factors have been shown to alter drug metabolism.¹⁴ However, with regard to disease status and exposure to drugs, our volunteers were a much more homogeneous group than the usual patient population.

Isoflurane was selected as a tracer gas because it undergoes little or no biotransformation, and because its solubility is comparable to that of halothane. The presence of isoflurane probably did not affect the metabolism of halothane. Less than one-thousandth the concentration of isoflurane necessary to produce anesthesia was present when concentrations of halothane reached K_m . Also, our estimates agree with published estimates (determined *in vitro*¹⁰ and *in vivo*^{9,10}) of the kinetics of halothane metabolism.

The values for K_m and V_{max} help clarify some confusing aspects of the literature concerning the metabolism of halothane. We⁸ and other investigators⁹ have demonstrated that the fractional metabolism of halothane decreases as its concentration increases; that is, as the inspired or end-tidal concentration rises, a greater fraction of the administered dose of halothane can be re-

TABLE 1. Residual Sums of Squares from the Fits to Each of the Model Equations

| Volunteer | General Model | Submodel 1 | Submodel 2 | Submodel 3 |
|-----------|---------------|------------|------------|------------|
| 1 | 304.3 | 321.9 | 323.8 | 2011.4 |
| 2 | 140.3 | 144.4 | 214.1 | 1737.5 |
| 3 | 109.8 | 133.5 | 228.5 | 1339.6 |
| 4 | 161.3 | 182.2 | 317.8 | 1351.5 |
| 5 | 196.2 | 214.9 | 340.1 | 937.5 |
| 6 | 253.1 | 253.1 | 433.1 | 2030.7 |
| 7 | 403.7 | 403.7 | 733.3 | 1071.1 |
| 8 | 111.2 | 130.8 | 336.4 | 1427.0 |
| 9 | 198.8 | 284.8 | 206.6 | 2107.0 |
| 10 | 413.8 | 414.0 | 786.5 | 2078.4 |
| TOTAL | 2293.0 | 2483.8 | 3920.8 | 16092.0 |

The total residual sum of squares is least for the general model (four parameters) but does not indicate a significantly better fit than the simpler submodel 1 (three parameters). However, submodel 1 does provide a significantly better fit than either of the other submodels.

claimed unchanged in the exhaled air or hepatic effluent. Since all anesthetic concentrations of halothane are many times the K_m , they all fuel metabolism at levels close to the maximum rate (V_{max}). Thus, doubling the anesthetic level, which may require twice the dose of halothane, will result in no additional metabolism during the anesthetic itself. Therefore, the fraction of the total dose that is metabolized will decrease.

However, higher concentrations of halothane do result in greater depot stores (especially muscle and fat), which during recovery will slowly release their contents of halothane at low concentrations when metabolic clearance is a major portion of total clearance. Thus, for anesthetics of equal duration, the higher the concentration of halothane, the greater the total amount of metabolism or metabolites generated. This increase in total metabolism that occurs during the recovery period is of insufficient magnitude to offset the decrease in fractional metabolism, because during the anesthetic period, total uptake always grossly exceeds V_{max} . Therein lies the paradox: that higher concentrations of halothane result in a decrease in the fractional metabolism but an increase in the total or absolute metabolism.

Metabolism during the recovery period should not be considered insignificant. On the contrary, for all clinically useful concentrations of halothane, our estimates of K_m and V_{max} allow us to predict that the wash-out period is of sufficient length and concentration to allow more metabolism in absolute terms to occur during recovery than during the anesthetic.

The significance of this work is threefold. First, we demonstrated a noninvasive method for measuring the kinetic parameters of metabolism of a volatile anesthetic. This method may be applicable to other inhalational anesthetics and, indeed, to most inhaled chemicals. Second, our technique permits the study of the effects of drugs (*e.g.*, common perioperative medications) on the metabolism of halothane or other inhaled anesthetics. Given the well-documented association of anesthetic metabolism and toxicity, we hope to use this model to investigate mechanisms of toxicity. Third, the values for K_m and V_{max} suggest that during the initial minutes of

recovery, halothane metabolism is at or near its maximum rate, and that the bulk of halothane metabolism occurs not during induction and maintenance, but during recovery.

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