

The Effects of Deuteration on the Metabolism of Halogenated Anesthetics in the Rat

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The authors studied the effects of substituting deuterium for hydrogen in several volatile anesthetics on their metabolism in the Fischer rat. Substitution of deuterium in the ethyl portion of methoxyflurane increased the metabolic production of fluoride ion by 19 per cent when administered at a concentration of 0.05 per cent. Total replacement of hydrogen by deuterium resulted in a 29 per cent decrease in the amount of fluoride produced, while deuteration of only the methoxyl group produced a 33 per cent decrease in fluoride produced. Deuteration of halothane resulted in a 15 or 26 per cent decrease in serum bromide at 0.75 per cent or 1.0 per cent, respectively. Deuteration in the ethyl portions of enflurane and two experimental agents, $\text{CF}_2\text{HOCF}_2\text{CFBrH}$ and $\text{CF}_2\text{HOCF}_2\text{CCl}_2\text{H}$ resulted in 65, 76, and 29 per cent decreases in urinary fluoride, respectively. Anesthesia with deuterated chloroform at a concentration of 0.36 per cent produced a 35 per cent decrease in serum glutamic pyruvic transaminase (SGPT). It is concluded that deuteration of volatile anesthetics changes their metabolism, in most cases producing decreases in metabolism. This effect may lessen the organ toxicity believed to occur with some of these anesthetics. (Key words: Anesthetics, volatile: methoxyflurane; halothane; enflurane. Metabolism: deuteration. Biotransformation (drug).)

BECAUSE HEPATOTOXICITY¹⁻² AND NEPHROTOXICITY³ may be related to the metabolism of volatile anesthetics, it would appear beneficial to decrease the metabolism of these drugs and thus lessen the amount of unwanted metabolic products. The replacement of hydrogen by deuterium in anesthetics appears to be a practical approach to the problem.

Materials and Methods

Groups of male Fischer 344 rats (200-300 g), with no pretreatment, were exposed to anesthetics in a 30-l glass exposure chamber. The anesthetic vapor was generated by metering the liquid compound at a controlled rate into a temperature-regulated (150 C) vaporization flask. The vapor was swept into the air inlet of the exposure chamber at a rate of 6 l/min. The concentration of the anesthetic in the exposure chamber was monitored by gas-liquid chromatography using direct gas sampling loops.

With various anesthetics, different analyses were made to assess metabolism and the effect of deutera-

tion. These were: 1) urinary inorganic fluoride and urinary volume; 2) serum bromide; 3) serum glutamic pyruvic transaminase (SGPT) as a measure of hepatic damage. Because there were minor variations in the conditions of the experiment (variations in the ages and weights of the animals), a control group that was not exposed to any anesthetic was included in each experiment. Thus, comparisons are valid only within each experiment.

Urinary fluoride was determined with an Orion® fluoride electrode similar to that described by Frant and Ross.⁴ The directions of the manufacturer were followed. Specifically, total ionic strength adjustor (containing glacial acetic acid, sodium chloride and cyclohexylene dinitrilo acetic acid) from Orion was used to prepare fluoride standards and also added to the urine samples. Glass containers were not used when fluoride measurements were being made. Bromide was measured with an Orion bromide electrode using the method of Poser, Poser and Müller-Oerlinghausen.⁵ Blood for serum bromide determinations was collected by decapitating the animals and allowing it to clot. The same serum collection method was used for SGPT determinations (Wróblewski and La Due⁶). The determinations were made with a CentrifChem® (Union Carbide).

The deuterated analogs of the anesthetics (table 1), except for chloroform, were prepared in the Halogens Research Laboratory of The Dow Chemical Company. The substitution of deuterium for a given hydrogen was more than 90 per cent for every compound except $\text{CD}_3\text{OCF}_2\text{CCl}_2\text{H}$, for which it was 72 per cent, the remaining 28 per cent being d_4 -MOF. The isotopic purity of each compound was established by nuclear magnetic resonance spectrometry and mass spectrometry. The deuterated chloroform, 99.8 atom per cent, was purchased from Merck and Company.

The data were processed by a one-way analysis of variance using a log transformation. Individual comparisons were done using the Neuman-Keuls test. Thus, data obtained from deuterated analogs could be compared with those from non-deuterated compounds, as well as non-anesthetized controls. Differences were judged significant when $P < 0.05$.

Results

For methoxyflurane, deuteration of a single hydrogen (d_1 -MOF) caused a 19 per cent increase in total

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fluoride excretion as compared with MOF (table 2). When d_4 -MOF was used, there was a 29 per cent decrease in total urinary fluoride, while d_3 -MOF caused a 33 per cent decrease in total urinary fluoride.

Anesthesia with deuterated enflurane produced a 65 per cent decrease in the fluoride produced by metabolism when compared with the non-deuterated analog. The effect of deuteration was most striking for the experimental anesthetic DFR, with which there was a 76 per cent decrease in urinary fluoride. DFM, which contains less fluorine in the molecule than does DFR, showed a 29 per cent decrease in urinary fluoride when the deuterated analog was used. The data also indicate that DFM is quickly cleared from the body, because urinary volume and urinary fluoride excretion during the second 24-hour period were not significantly different from control values.

For deuterated halothane, serum bromide values were 15 per cent lower than those for halothane when the anesthetic concentration was 0.75 per cent (table 3). Increasing the halothane concentration to 1 per cent produced a 26 per cent lower value of serum bromide for deuterated halothane when the same agents were compared. The experimental compound, DFR, gave a serum bromide level almost twice that of the deuterated analog.

Chloroform anesthesia produced hepatotoxicity, as indicated by an increase in SGPT. Rats anesthetized with chloroform at a concentration of 0.36 per cent for two hours had SGPT $56.3 \text{ mU/ml} \pm 24$ when

TABLE 1. Anesthetics and Their Deuterated Analogs Used in This Study

Anesthetic	Deuterated Forms Studied	Duration of Exposure (Hours)
Methoxyflurane (MOF) $\text{CH}_3\text{OCF}_2\text{CCl}_2\text{H}$	$\text{CH}_3\text{OCF}_2\text{CCl}_2\text{D}$ (d-MOF)	2
	$\text{CD}_3\text{OCF}_2\text{CCl}_2\text{H}$ (d_3 -MOF)	2
	$\text{CD}_3\text{OCF}_2\text{CCl}_2\text{D}$ (d_4 -MOF)	2
Halothane (H) CF_3CClBrH	CF_3CClBrD (d-H)	2
Enflurane (E) $\text{CF}_2\text{HOCF}_2\text{CFCIH}$	$\text{CF}_2\text{HOCF}_2\text{CFCID}$ (d-E)	3
Difluororoflurane* (DFR) $\text{CF}_2\text{HOCF}_2\text{CFBrH}$	$\text{CF}_2\text{HOCF}_2\text{CFBrD}$ (d-DFR)	3
Difluoromethoxyflurane* (DFM) $\text{CF}_2\text{HOCF}_2\text{CCl}_2\text{H}$	$\text{CF}_2\text{HOCF}_2\text{CCl}_2\text{D}$ (d-DFM)	2
Chloroform CHCl_3	CDCl_3	2

* The names difluororoflurane and difluoromethoxyflurane are assigned only for the purpose of identification and are not official generic names.

sacrificed at 24 hours. Control animals had a value of $24.8 \text{ mU/ml} \pm 2$; animals anesthetized with deuterated chloroform, $38.3 \text{ mU/ml} \pm 7$. Thus, the value for animals treated with deuterated chloroform

TABLE 2. A Comparison of Various Deuterated Anesthetics with the Non-Deuterated Analogs

Anesthetic Agent	Concentration (Per Cent)	n	Urinary Volume, ml (Mean $\pm$ SD)		Urinary F^- , $\mu\text{mol} \times 10^{-1}$ (Mean $\pm$ SD)		Total Urinary F^- , (nmol $\times 10^{-1}$) (Mean $\pm$ SD)
			24 Hours	48 Hours	24 Hours	48 Hours	
Control	—	7	9.4 ± 2.1	8.6 ± 1.5	1.0 ± 0.2	0.8 ± 0.4	16.5 ± 5.6
MOF	0.5	7	13.0 ± 3.8	8.8 ± 1.9	8.4 ± 2.8	6.7 ± 2.1	171.6 ± 32.3
d -MOF	0.5	7	12.3 ± 2.3	8.6 ± 1.6	12.1 ± 1.5	7.5 ± 1.4	$210.5 \pm 34.8^*$
Control	—	6	10.3 ± 3.2	10.6 ± 2.9	1.4 ± 0.3	1.6 ± 0.3	30.4 ± 3.6
MOF	0.5	6	15.1 ± 5.2	11.6 ± 3.2	7.8 ± 1.9	7.1 ± 1.6	187.8 ± 18.8
d_4 -MOF	0.5	6	14.2 ± 5.5	11.8 ± 2.7	$5.5 \pm 1.7^*$	$5.5 \pm 1.4^*$	$133.7 \pm 21.2^*$
Control	—	6	9.1 ± 2.6	5.6 ± 1.3	1.3 ± 0.3	1.8 ± 0.8	20.8 ± 5.0
MOF	0.5	6	12.3 ± 5.1	5.2 ± 1.7	14.1 ± 3.3	12.6 ± 1.9	226.3 ± 55.0
d_3 -MOF	0.5	6	15.8 ± 2.5	$11.0 \pm 2.0^*$	$7.0 \pm 2.7^*$	$3.7 \pm 1.8^*$	$149.5 \pm 52.0^*$
Control	—	8	9.2 ± 0.8	10.9 ± 2.6	0.9 ± 0.4	1.1 ± 0.2	20.6 ± 2.7
E	2.5	8	14.8 ± 2.2	11.0 ± 1.9	4.3 ± 0.7	1.6 ± 0.2	80.7 ± 10.1
d-E	2.5	8	$10.9 \pm 1.5^*$	$8.7 \pm 1.5^*$	$1.7 \pm 0.2^*$	$1.1 \pm 0.1^*$	$28.6 \pm 4.7^*$
Control	—	6	9.1 ± 1.0	8.5 ± 1.5	0.7 ± 0.1	0.8 ± 0.1	12.7 ± 0.7
DFR	1.5	4	12.8 ± 1.4	8.0 ± 1.5	10.4 ± 1.9	3.3 ± 0.7	156.8 ± 20.9
d-DFR	1.5	4	11.3 ± 1.9	7.4 ± 1.0	$2.6 \pm 0.9^*$	$1.3 \pm 0.1^*$	$37.8 \pm 5.8^*$
Control	—	7	10.2 ± 2.5	8.3 ± 2.8	0.7 ± 0.0	0.8 ± 0.1	14.2 ± 3.4
DFM	0.5	7	14.6 ± 2.3	10.0 ± 2.1	1.4 ± 0.2	1.0 ± 0.1	30.2 ± 3.6
d-DFM	0.5	7	$12.2 \pm 1.3^*$	9.6 ± 1.6	1.1 ± 0.1	0.8 ± 0.1	$21.5 \pm 2.9^*$

* Significant difference, $P < 0.05$.

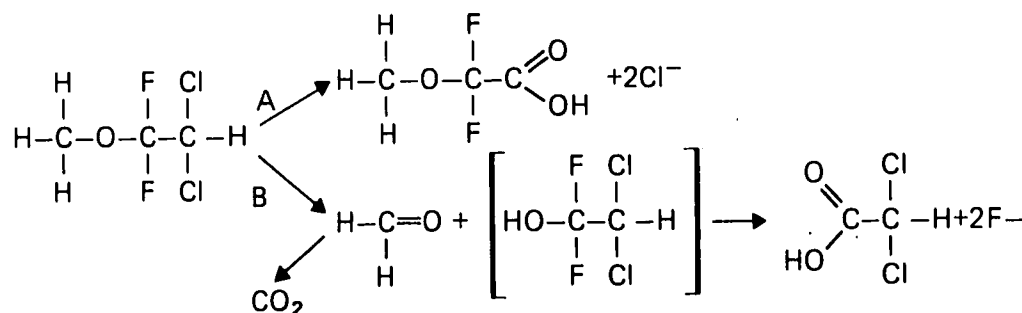


FIG. 1. A metabolic scheme for methoxyflurane proposed by Holaday *et al.*⁷ (Reproduced by permission of the authors and publisher.)

was 35 per cent less than that for those treated with chloroform. This difference is statistically significant.

Discussion

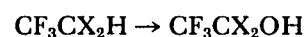
The metabolism of methoxyflurane has been shown by Holaday *et al.*⁷ to proceed by two pathways (fig. 1). Assuming this scheme is correct, then deuterating the ethyl portion of the molecule would slow the metabolic attack on it and allow more metabolism to proceed via path B. Deuterating both ends of the molecule would slow overall metabolism, resulting in a decrease in urinary fluoride. Deuteration of the methoxyl group would allow metabolism to proceed preferentially via path A, resulting in a further decrease in urinary fluoride. The results of our experiments are consistent with this hypothesis.

Cohen *et al.*⁸ have proposed three pathways for the metabolism of halothane (fig. 2). In our experiments, we measured only serum bromide and found it decreased when halothane was deuterated. Since the major metabolite of halothane is thought to be trifluoroacetic acid, deuteration should have an effect on the kinetics involved in the abstraction of HBr.

The same may be true of the removal of HF, but we have not yet measured the influence of deuteration on this metabolic step.

A metabolic scheme for enflurane has been proposed by Cousins and Mazze.⁹ Quantitative measurements to show that one pathway is preferred over the other have not been made. If, as suggested by Hitt, *et al.*,¹⁰ the difluoromethoxy structure is more resistant to metabolism than the methoxyl group of methoxyflurane, then difluoromethoxydifluoroacetic acid and fluoride ion would be the major metabolites. Thus, deuteration of the ethyl portion of enflurane decreases metabolic production of fluoride. A similar explanation can be used in the interpretation of the results obtained by the deuteration of CF₂HOCF₂CBrFH. In the case of CF₂HOCF₂CCl₂H (D), this argument cannot explain the effect of deuteration. The pathway proposed by Hitt *et al.* theoretically should result in release of little or no fluoride. However, some fluoride does appear in the urine.

The first step in the metabolism of anesthetics has been postulated to be the insertion of oxygen.



Oxygen insertion would involve breaking a CH or a CD bond. Because the CD bond has greater stability, the deuterated analog would be more resistant to metabolic attack. This metabolic scheme would explain all the results we obtained except those with the experimental compound CF₂HOCF₂CCl₂D, (d₁-DFM).

In the case of DFM, metabolic attack on the ethyl portion of the molecule by oxidation would produce CF₂HOCF₂COOH, as proposed by Cousins and Mazze.⁹ This, however, would not yield fluoride as a metabolic product. Therefore, the fluoride produced by the metabolism of this compound must come from attack on the difluoromethyl or the 1,1-difluoro portion of the molecule, or both. Deuteration of the ethyl portion would do nothing to protect the difluoromethyl from metabolic attack. Since our results cannot be ex-

TABLE 3. Serum Bromide Values of Rats Anesthetized with Deuterated and Non-Deuterated Anesthetics

Anesthetic Agent	Concentration (Per Cent)	n	Serum Br ⁻ (mEq/l) (Mean ± SD)	Time of Serum Collection
Control	—	6	0.52 ± 0.02	72 hours
H	0.75	6	1.90 ± 0.10	72 hours
H-D	0.75	6	1.60 ± 0.10*	72 hours
Control	—	6	0.24 ± 0.02	24 hours
H	1.0	6	1.36 ± 0.17	24 hours
d-H	1.0	6	1.00 ± 0.07*	24 hours
Control	—	6	0.53 ± 0.02	48 hours
DFR	1.5	4	1.59 ± 0.70	48 hours
d-DFR	1.5	4	0.82 ± 0.09*	48 hours

* Significant difference, $P < 0.01$.

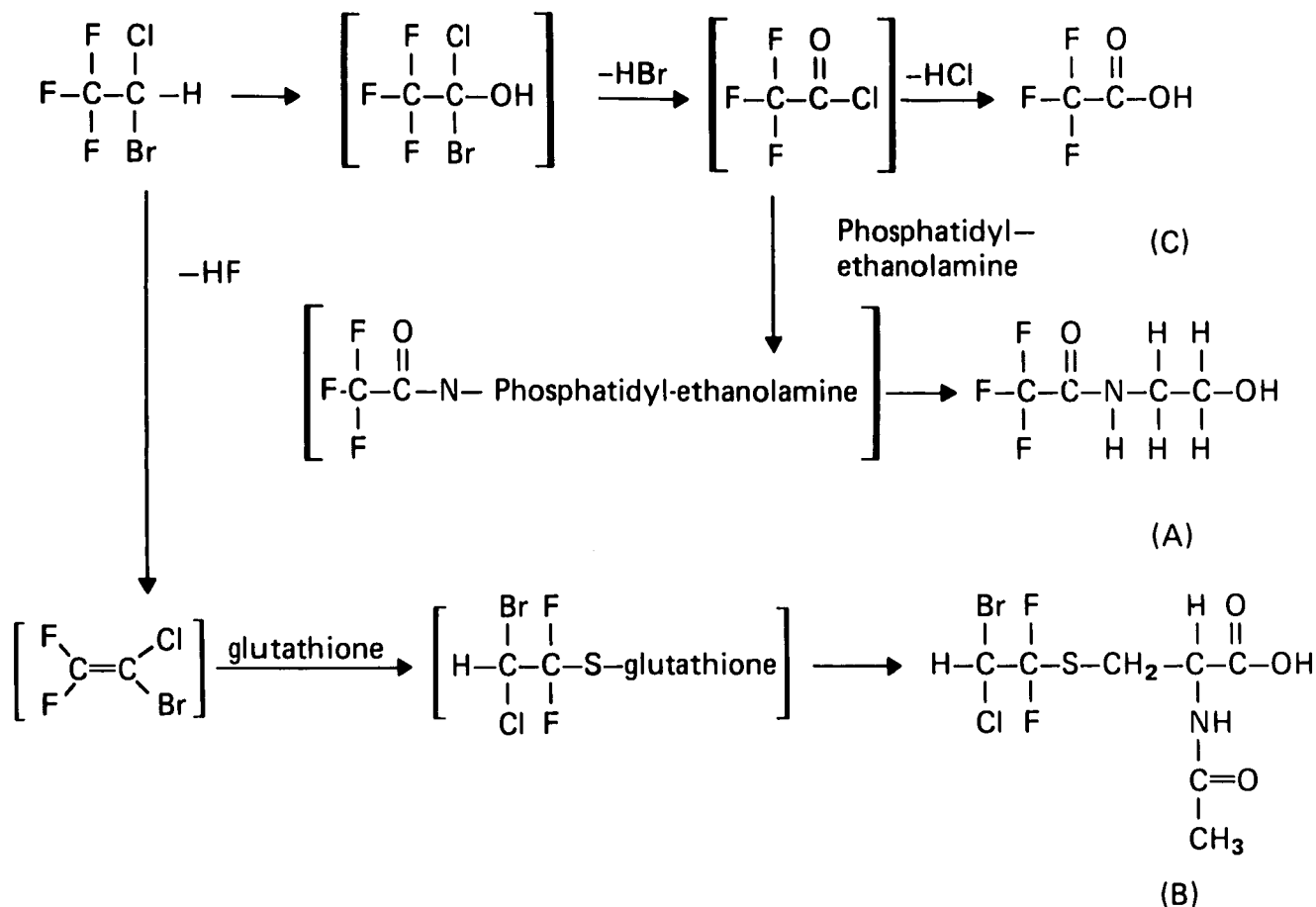


FIG. 2. A metabolic scheme for halothane proposed by Cohen *et al.*⁸ (Reproduced by permission of the authors and publisher.)

plained by the oxidative pathways involving hydroxyl intermediates, others should be considered.

Two metabolites of halothane, CF_2CHCl and $\text{CF}_3\text{CH}_2\text{Cl}$, have been demonstrated in rabbits by Mukai *et al.*¹¹ These same metabolites have been identified in human beings after anesthesia with halothane by Sharp *et al.*¹² These investigators have suggested two types of reactions leading to the metabolites they isolated. One of the mechanisms involves the production of a carbanion with the loss of a bromide ion. The second reaction involves reduction with the loss of a bromide ion. The use of deuterated halothane should not have an effect on either of these reactions. On the other hand, if a proton or deuteron were the first step in the reaction sequence, then the carbanion, $\text{CF}_3\text{CClBr}^-$, could be formed. Hine *et al.*¹³ showed this reaction takes place under base catalysis and does show a deuterium effect. If the carbanion lost fluoride ion, it would become CF_2CClBr and be the source of the glutathione adduct isolated by Cohen *et al.*⁸ (fig. 2).

Using the same sequence, $\text{CF}_2\text{HOCF}_2\text{CCl}_2\text{H}$ could lose a proton to become the carbanion $\text{CF}_2\text{HOCF}_2\text{CCl}_2^-$. This in turn could be rearranged to form $\text{CF}_2\text{HOCFCCL}_2$ and a fluoride ion. The resulting alpha-beta unsaturated ether would be a very unstable compound, and the whole molecule would disintegrate. Using this hypothesis, it is possible to explain the metabolic production of fluoride and the effect of deuteration observed when the compound $\text{CF}_2\text{HOCF}_2\text{CCl}_2\text{H}$ (D) is used as an anesthetic.

In conclusion, the substitution of deuterium for hydrogen in anesthetics changes the metabolism of these agents significantly. In most cases, the deuteration would appear to decrease the production of metabolites associated with the toxic side effects of anesthetics. Assuming this is true, deuterated anesthetics may become important in the clinical practice of anesthesia.

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