Metabolism of Halothane-2 ^{14}C in the Mouse

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The intravenous injection of halothane-2 ^{14}C into the mouse was followed by an accumulation of labeled nonvolatile metabolites, with the highest concentrations present in the liver. These materials remained in the body for at least 12 days, although in decreasing amounts. Comparative studies using other ^{14}C-labeled anesthetics indicated decreasing ratios of liver-to-whole-body radioactivity for diethyl ether and chloroform, but an increasing ratio for halothane. Repeated injection of halothane at weekly intervals for five weeks yielded a rapidly-increasing concentration of radioactivity in the liver, suggesting the stimulation of enzyme-induction systems. Implications of these findings are discussed. (Key words: Halothane; Metabolism; Liver.)

RECENT low-temperature autoradiographic studies with labeled halothane in the mouse have demonstrated the accumulation of nonvolatile metabolite(s) in the liver.[1] Investigations of anesthetic metabolism by Stier[2] and Rehder[3] previously indicated the prolonged excretion of trifluoroacetic acid and bromide in the urine of patients given halothane.

Although there is no firm evidence linking the administration of halothane to hepatic necrosis, data suggesting that repeated administration of halothane anesthesia may carry an increased hazard have been accumulated.[4, 5, 6] Among the possible explanations for this finding is that of accelerated enzymic activity, producing a toxic accumulation of those products into which enzymes help transform halothane.[7]

The present study was designed to investigate the extent of halothane metabolism in the mouse following a single intravenous injection of labeled anesthetic, and to evaluate further the effect of repeated injections upon the accumulation of metabolites and upon stimulation of enzyme-induction systems.

Procedure

Halothane-2 ^{14}C, specific activity 1.2 mC/mM, was obtained from commercial sources.† Radio gas chromatography established the material to be of high purity. The labeled halothane was diluted with cold halothane and 4 μC (approximately 7.44 mg) injected into the tail vein of each of 70 Swiss white mice, averaging 20 gm in weight at the time of the initial experiment. The animals were divided into two groups for study purposes.

The animals in Group I were given a single injection of 4 μC of labeled halothane and sacrificed at intervals of two and four hours and one, two, three, five, eight, and 12 days after administration of the anesthetic. All animals were fed mouse pellets and water *ad lib.* during the course of the experiment. After sacrifice, the mice were reweighed, the livers removed and weighed separately. Following addition of an equal volume of distilled water, each carcass (less the liver) was homogenized in an omnimixer.‡ Several biopsies taken from the liver and aliquots of homogenized tissue were then dried to remove any remaining volatile radioactivity. These were solubilized in 1.0 ml N.C.S. solution § and ten ml of toluene cocktail ¶ added to each vial prior to scintillation counting.

In Group II, the animals were given weekly injections of 4 μC of labeled halothane for periods as long as five weeks. The animals were sacrificed at intervals of seven, 14, 21, 28, and 35 days, the seven-day animals having one injection, the 14-day animals two injections, etc. Since repeated injections of halothane into the

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‡ Ivan Sorvall Inc., Norwalk, Connecticut.

§ Amersham/Searle, Des Plaines, Illinois.

¶ 2,5-diphenyloxazole; 5 gm/l toluene.

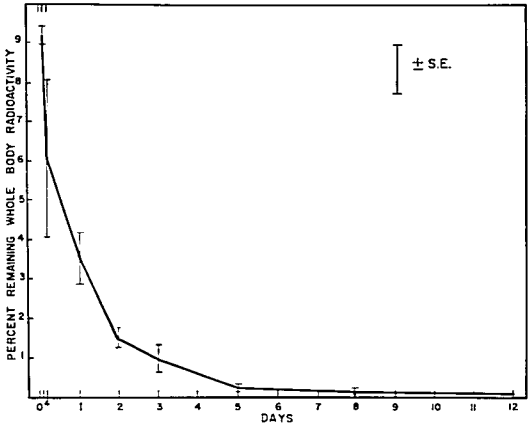

FIG. 1. Per cent of whole-body radioactivity (nonvolatile metabolites) remaining following a single intravenous injection of 4 μC halothane-2 ^{14}C.

tail vein frequently produced tissue slough, it was necessary to begin the initial study with an excess number of animals. After sacrifice, the animals in Group II were treated like those in Group I.

A comparative study was made using two other ^{14}C-labeled volatile anesthetics. Four μC of ^{14}C-labeled chloroform were injected into each of 12 mice, and the animals sacrificed at one, three, eight, and 12 days. Another 18 mice were given 4 μC of 1-^{14}C diethyl ether and sacrificed at one, two, three, five, eight, and 12 days. In all other respects

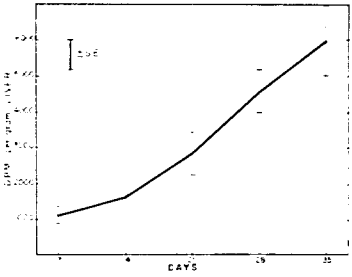

FIG. 2. Concentration of nonvolatile metabolites accumulating in the liver following repeated weekly injections of 4 μC halothane-2 ^{14}C.

TABLE 1. Percentages of Nonvolatile Metabolites Remaining in the Body Following Intravenous Injection of 4 μC Halothane-2 ^{14}C

Time of Sacrifice	d.p.m./Mouse*	Per Cent of Radioactivity
2 hours	809,667±33,525 (3)	9.2±0.4
4 hours	546,333±191,646 (3)	6.2±2.2
1 day	303,193±59,148 (6)	3.4±0.7
2 days	136,633±19,283 (3)	1.6±0.2
3 days	106,020±37,522 (5)	1.2±0.4
5 days	25,232±9,136 (6)	0.2±0.08
8 days	3,309±2,087 (4)	0.04±0.02
12 days	304±10 (2)	0.003±0.0001

* Disintegrations per minute ± SE. Number of animals in each experiment indicated in parentheses.

TABLE 2. Concentrations of Nonvolatile Metabolites Remaining in the Liver Following Repeated Weekly Injections of 4 μC Halothane-2 ^{14}C

Day of Sacrifice	No. of Injections	d.p.m./Mouse Liver*
7	1	1,129±224 (7)
14	2	1,609±104 (7)
21	3	2,290±637 (8)
28	4	4,657±673 (8)
35	5	5,902±1,204 (8)

* Disintegrations per minute ± SE. Number of animals in each experiment indicated in parentheses.

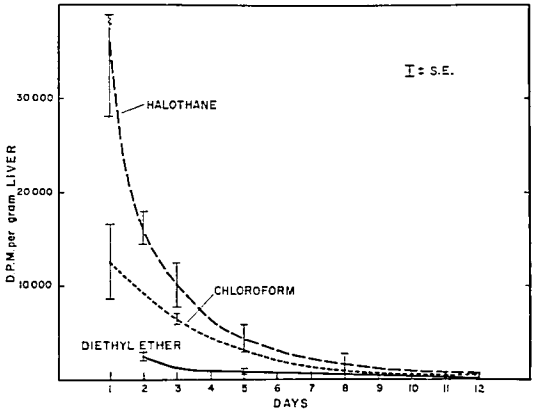

FIG. 3. Concentration of nonvolatile metabolites accumulating in the liver following a 4 μC injection of ^{14}C chloroform, halothane, or diethyl ether.

the animals in these two groups were treated like the Group I halothane series.

Results

Following the intravenous injection of halothane-2 ^{14}C, a peak concentration of accumulated nonvolatile metabolite(s), equivalent to 9.2 per cent of the injected radioactivity, was present within two to four hours. By 24 hours, 3.4 per cent of the total injected radioactivity was still present in the body. The concentration of the nonvolatile metabolites decreased slowly, and traces remained 12 days after anesthetic administration (fig. 1, table 1).

TABLE 3. Concentrations of Nonvolatile Metabolites Remaining in the Body Following Intravenous Injection of 4 μC Labeled Halothane, Chloroform, and Diethyl Ether

Anesthetic	Day of Sacrifice	d.p.m./Mouse*	d.p.m./Liver	Per Cent of Total Body Radioactivity in Liver
Halothane	1	303,193±59,149 (6)	35,910±8,383	11.54±0.93
	2	136,633±19,283 (3)	15,633±1,530	11.51±0.56
	3	106,020±37,522 (5)	11,520±2,773	13.68±2.34
	5	25,232±9,136 (6)	4,598±1,500	20.94±3.40
	8	3,309±2,087 (4)	1,042±585	33.20±5.05
	12	304±10 (2)	—	—
Chloroform	1	35,647±11,493 (3)	12,460±4,133	34.33±1.91
	3	19,660±1,843 (3)	6,360±507	32.50±1.35
	8	4,797±2,212 (3)	687±388	14.38±3.09
	12	3,223±810 (3)	388±62	13.12±3.21
Diethyl ether	1	17,560±3,112 (3)	1,530±361	8.52±0.99
	2	28,497±337 (3)	1,963±296	6.89±1.03
	3	17,370±5,310 (3)	937±125	5.91±0.85
	5	18,326±3,578 (3)	776±182	4.27±0.53
	8	13,994±5,955 (3)	417±148	3.55±0.90
	12	9,483±3,040 (3)	207±65	2.21±0.04

* Disintegrations per minute ± SE. Number of animals in each experiment indicated in parentheses.

Administration of halothane repeated at weekly intervals yielded increasing accumulations of nonvolatile metabolites in the liver. Over a five-week period there was a 423 per cent increase in nonvolatile radioactivity in the liver compared with the amount present seven days after the first injection. Larger incremental changes resulted from succeeding injections (fig. 2, table 2).

A comparative study of anesthetic metabolism following single injections of ^{14}C-labeled halothane, chloroform, and diethyl ether indicated that halothane had the highest rate of production of nonvolatile metabolites. Reference to figure 3 and table 3 further suggests that a high concentration of the nonvolatile halothane metabolites tended to remain in the body for prolonged periods of time. In contrast, a lesser amount of nonvolatile metabolites accumulated following diethyl ether anesthesia. Chloroform occupied an intermediary position.

An analysis of the percentages of nonvolatile metabolites accumulating in the liver indicates that the ratio of radioactivity remaining in the liver to that in the whole body falls rapidly with chloroform and more slowly with diethyl ether. With halothane, on the other hand, there is a threefold increase in the ratio of nonvolatile metabolites present in the liver (fig. 4). On a percentage-of-body-weight basis, the liver in eight days contains five times the concentration of nonvolatile metabolites present in other body tissues. Autoradiographic figure 5 graphically illustrates the slow elimination of radioactivity from the body and the high relative concentration of nonvolatile metabolites accumulating in the liver and large intestine following halothane anesthesia. The specimen in figure 5 was prepared from an animal sacrificed eight days after intravenous administration of 4 μC halothane-2 ^{14}C anesthesia.*

Discussion

Considerable controversy remains about the question of hepatic injury following expo-

* Autoradiographs were prepared from thin whole-body sections (40 μ) taken to dryness and heated to 80 C for four hours. Since only a relatively small amount of radioactivity remains in the eight-day animal, photographic exposure time has been increased proportionately.

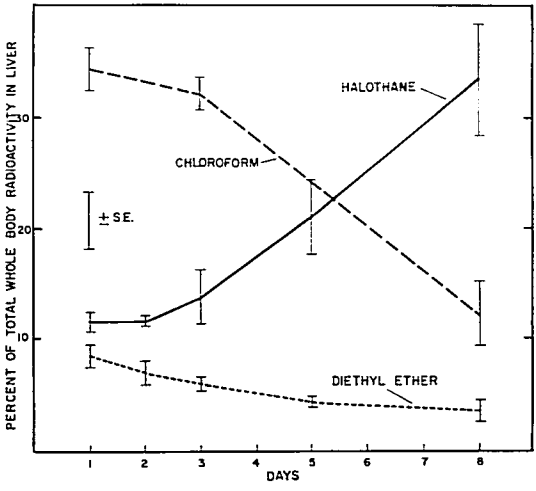

FIG. 4. Per cent of the total whole-body radioactivity present in the liver following a 4 μC intravenous injection of ^{14}C chloroform, halothane, or diethyl ether.

FIG. 5. Autoradiograph (40-μ section) from a mouse sacrificed eight days after injection of 4 μC halothane-2 ^{14}C. Radioactivity indicates the presence of nonvolatile metabolites. 1, liver; 2, large intestine.

sure(s) to halothane. Almost 400 cases of hepatic damage after halothane anesthesia were reported in the literature prior to 1968.[3] The National Halothane Study estimated an overall death rate from massive hepatic necrosis following halothane anesthesia at 1 in 10,000.[4] A recent editorial has suggested, "The current status of halothane liver damage has gone beyond the point of whether such an entity exists; it is now necessary to obtain information about the frequency of this complication."[9] Nonetheless, the overall incidence of hepatic complications following halothane remains low and is difficult to evaluate statistically. In addition, many nonanesthetic factors can be shown to contribute as independent causes of hepatic necrosis. Finally, other anesthetics have also been shown to be associated with hepatic necrosis.[4]

The differences observed between accumulation of nonvolatile metabolites after administration of certain of the anesthetic agents probably reflect their various routes of metabolism. For example, diethyl ether is largely carried to $^{14}CO_2$, which in turn serves as a source of carbon which can be incorporated into naturally-occurring body materials, *i.e.*, fatty acids, cholesterol, etc. The halothane-2 ^{14}C, on the other hand, maintains its label attached to fluorine as $^{14}CF_3$, representing a foreign radical to be excreted (conjugated?) by the liver.[1]

Although some investigators have suggested that halothane induces hepatic injury as the result of a hypersensitivity reaction rather than by direct hepatotoxicity,[5, 10] there is no evidence for formation of a halothane antigen known to produce this sensitivity type of response. Therefore, interest in the possible role of halothane metabolites as causative factors has been stimulated. The present study indicates not only a prolonged retention in the body of certain nonvolatile halothane by-products but, following repeated weekly injection of halothane, a markedly increased level of these materials develops. This increase in concentration is of a magnitude suggesting an independent mechanism, such as stimulation of enzyme-induction systems. Finally, it is of interest that in addition to an initial high concentration of nonvolatile materials in the liver, the ratio of liver-to-whole-body radioactivity further increases significantly with time following halothane-2 ^{14}C anesthesia.

As suggested, it is of critical importance to identify these materials precisely in order to determine their possible hepatotoxicity.[1] It is possible, of course, that the nonvolatile metabolites demonstrated in these studies are species variants or entirely harmless products. The earlier-mentioned suspicions remain, however, impelling us to investigate thoroughly the accumulation of any foreign materials in the liver. Further studies are under way.

References

1. Cohen, E. N., and Hood, N.: Application of low-temperature autoradiography to studies of the uptake and metabolism of volatile anesthetics in the mouse. III. Halothane, ANESTHESIOLOGY 31: 553, 1969.
2. Stier, A., and Alter, H.: Stoffewechselprodukte des Halothane in Urin, Anaesthesist 15: 154, 1966.

3. Rehder, K., Forbes, J., Alter, H., Hessler, O., and Stier, A.: Halothane biotransformation in man, a quantitative study, ANESTHESIOLOGY 28: 711, 1967.
4. Bunker, J. P., *et al.*: Summary of the National Halothane Study. Subcommittee on the National Halothane Study of the Committee on Anesthesia, National Academy of Sciences–National Research Council, J.A.M.A. 197: 775, 1966.
5. Trey, C., Lipworth, L., Chalmers, R. C., Davidson, C. S., Gottlieb, L. S., Popper, H., and Saunders, S. J.: Fulminant hepatic failure. Presumable contribution of halothane, New Eng. J. Med. 279: 798, 1968.
6. Klatskin, G., and Kimberg, D. V.: Recurrent hepatitis attributable to halothane in an anesthetist, New Eng. J. Med. 280: 515, 1969.
7. Artusio, J. F.: Discussion. Liver failure after repeated use rekindles halothane safety debate, J.A.M.A. 207: 2197, 1969.
8. Greene, N. M. (ed.): Halothane. *In* Clinical Anesthesia Series' 1/1968. Philadelphia, F. A. Davis, 1968.
9. Combes, B.: Halothane induced liver damage—an entity (editorial), New Eng. J. Med. 280: 558, 1969.
10. Klatskin, G.: Symposium on toxic hepatic injury—clinical aspects, Gastroenterology 38: 789, 1960.

Surgery

HEPATITIS Fifty-five adult patients undergoing cardiopulmonary bypass were evaluated for hepatitis clinically and by determination of SGOT and SGPT every four weeks for six months following open-heart surgery. In 42 patients receiving mainly blood from paid donors, the combined attack rate for icteric and anicteric hepatitis was 60 per cent. In a second group of 18 patients receiving blood mainly (97 per cent) from voluntary donors, no cases of icteric or anicteric hepatitis were seen. (*Walsh, J. H., and others: Icteric and Anicteric Hepatitis Following Open-heart Surgery: A Direct Comparison of Paid and Voluntary Blood Donors, Transfusion 8: 318 (Sept.) 1968.*)

TRANSFUSION HEPATITIS Bayes' theorem may be helpful in estimating the probability that an individual blood donor is a hepatitis carrier. This theorem suggests that there exists a critical probability above which a prospective donor should be rejected. Decision theory also provides a mechanism for introducing and "blending" other information about a particular case of hepatitis so as to produce the best probability estimates. (*Polissar, J.: Transfusion Hepatitis: Use of Statistical Decision Theory, Transfusion 9: 15 (Jan.) 1969.*) ABSTRACTER'S COMMENT: This theorum might also be used to estimate the probability that poor-risk patients will survive anesthesia and surgery.

RECURRENT HALOTHANE HEPATITIS Recurrent bouts of hepatitis and the development of cirrhosis were observed in an anesthetist with a history of hay fever and asthma. Each relapse was coincident with the physician's return to work and reexposure to halothane. An identical relapse consisting of chills, fever and acute hepatitis occurred following a challenge inhalation of 0.1 to 0.2 per cent halothane in oxygen for five minutes. This relapse was documented both biochemically and histologically. These interesting observations support the authors' view that in a few uniquely susceptible persons, halothane is a sensitizing agent capable of evoking acute hepatitis and other manifestations of hypersensitivity. (*Klatskin, G., and Kimberg, D. V.: Recurrent Hepatitis Attributable to Halothane Sensitization in an Anesthetist, New Eng. J. Med. 280: 515 (March) 1969.*)