Volume 33, No. 6
December 1970

THE JOURNAL OF Anesthesiology
THE AMERICAN SOCIETY OF ANESTHESIOLOGISTS, INC.

The Metabolic Degradation of Methoxyflurane in Man

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The metabolism and excretion of methoxyflurane were studied in 12 human subjects, five of whom were exposed to methoxyflurane labeled with ^{14}C in the methoxy position. Biodegradation of methoxyflurane began immediately after the onset of exposure and continued for nine to 12 days, after which storage depots of intact drug approached depletion. Identified products of biotransformation were CO_2, fluoride ion, dichloroacetic acid, and methoxyfluoroacetic acid. Twenty-nine and 35 per cent of the absorbed methoxyflurane, respectively, were estimated to be exhaled unaltered by two subjects. Seven to 21 per cent underwent cleavage of the ether linkage, producing CO_2, fluoride ion and dichloroacetic acid. A larger fraction, 40 per cent in one subject, was dechlorinated and oxidized to methoxydifluoroacetic acid, which was excreted promptly by the kidney. (Key words: Metabolic degradation; Methoxyflurane.)

RECENT REPORTS have established that volatile organic anesthetic drugs are metabolized by vertebrate enzyme systems *in vivo*[1-3] and *in vitro*.[4] Van Dyke, Chenoweth and Van Posnak[1] have shown that ^{14}C-labeled diethyl ether, chloroform and methoxyflurane (Penthrane) are converted *in vivo* by the rat to $^{14}CO_2$ to the extent of 3 to 6 per cent of the amount injected. Following injection of ^{36}Cl-labeled halothane (Fluothane) and methoxyflurane, the recovery from urine of ^{36}Cl as inorganic chloride approximated 3 per cent of the injected doses. Eighty-five to 90 per cent of the injected methoxyflurane was recovered from exhaled air in 30 hours in unaltered form.

Trichloroethylene[5,6] and halothane[2,7] are the only volatile anesthetic drugs whose metabolic pathways in man have been reported. Soucek and Vlachova[6] found that an average of 73 per cent of trichloroethylene was excreted as urinary metabolites by man, 4 per cent as monochloroacetic acid, 19 per cent as trichloroacetic acid, and 50 per cent as trichloroethanol. Stier *et al.*[3] identified excess inorganic bromide ion in the urine of men following halothane anesthesia. Rehder and associates[7] determined, in two patients, that 11 and 12 per cent, respectively, of halothane absorbed during 75 minutes of exposure were converted to trifluoroacetic acid and bromide ion. Cascorbi, Blake and Helrich[8] recovered 10.6 to 24.8 per cent of ^{14}C-labeled urinary metabolites from nine volunteers, the amount apparently being influenced by prior chronic exposure to low concentrations of anesthetics. Evidence for significant metabolic degradation of methoxyflurane or cleavage of C—F bonds in man has not been reported.

The present studies were undertaken to determine the manner and extent of conversion

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Accepted for publication August 19, 1970. Supported by National Institutes of Health Grants NB 04212-04-05 and by grants-in-aid from Abbott Laboratories, Research Division, and from Ohio Medical Products.

of methoxyflurane to other compounds in man during and following clinical anesthesia. Expired air was examined for labeled carbon dioxide, and urine was examined for labeled carbon, free fluoride ion, nonvolatile fluorine-containing organic compounds, glucuronides and dichloroacetic acid.

Methods

Four surgical patients were observed for excretion of $^{14}CO_2$ following exposure to labeled methoxyflurane, and seven surgical patients who received unlabeled methoxyflurane were observed for renal excretion of fluoride ion; of the seven, five were also studied to determine the total amount of fluorine excreted (table 1). One volunteer not operated upon was given a precisely known quantity of ^{14}C-labeled methoxyflurane and was studied to determine the amount of $^{14}CO_2$ exhaled and urinary excretion of ^{14}C, total fluorine and fluoride ion. The urines of five patients who did not receive fluorinated drugs during anesthesia also were examined for fluoride ion and total fluorine content (table 1).

^{14}C-methoxyflurane, labeled in the methoxy position,¶ was diluted to 7.05 microcuries/ml and administered to each of four adult patients undergoing elective operations. The trachea was intubated with a cuffed, rubber endotracheal tube with the patient under thiopental (Pentothal), nitrous oxide and succinylcholine anesthesia prior to exposure to methoxyflurane. The methoxyflurane was delivered from a Pentec vaporizer into a nonrebreathing circuit constructed entirely of plastics and metal except for a 5-l conductive rubber storage bag mounted near the vaporizer. A flow of equal parts of oxygen and nitrous oxide was maintained in slight excess of the patient's minute volume. An estimate of the quantity of methoxyflurane absorbed by one of the four patients was obtained by sampling and analyzing inspired air and mixed expired air, and measuring the exhaled minute volume with a Wright respirometer at intervals of approximately ten minutes during administration. Similar measurements of rate of absorption were obtained at two and three points in time, respectively, in two of the other three studies.

One unpremedicated, healthy, informed, adult male volunteer (Subject 17) was exposed to 10 ml of labeled methoxyflurane containing a total of 66.4 microcuries of ^{14}C. The breathing circuit was entirely closed, and was constructed of brass, teflon, a polypropylene bellows, and 570.8 g of fresh water-saturated Baralyme. The gas volume of the circuit was 7.0 l. The drug was delivered into the circuit in liquid form at a rate calculated to maintain a blood concentration in the range of 10–12 mg/100 ml for an hour. The circuit was kept closed until the concentration of methoxyflurane in the inspired gas was 0.1 per cent and the subject awoke, which occurred two hours after the start of anesthesia.

Analysis of Exhaled Air

The quantities of methoxyflurane and $^{14}CO_2$ excreted in exhaled air were estimated from analyses of exhaled air and measurements of minute volume with a Wright respirometer at approximately hourly intervals following anesthesia on the day of operation, and two or three times a day on subsequent days. Exhaled air was collected by means of a nonrebreathing valve and mask system into laminated mylar bags, 15 liters in capacity, which previously had been tested and found to be impervious to methoxyflurane. Concentration of methoxyflurane was measured in a gas chromatograph equipped with a hydrogen flame detector (Microtek 2500R), and carbon dioxide was measured with a calibrated infrared CO_2 analyzer (Beckman Medical Gas Analyzer, Spinco Model LB-1). Arterial blood was sampled simultaneously with the gas collections on the day of anesthesia and was extracted with tetrachloroethylene for measurement of methoxyflurane content by gas chromatography.

Specific Activity of Exhaled CO_2

Measured volumes of expired gas samples were pumped through carbonate-free 1N sodium hydroxide, from which barium carbonate was precipitated with 3N $BaCl_2$. The precipitate was washed with dilute NaOH and

¶ Supplied through the courtesy of Dr. Norman Wheeler of Abbott Laboratories and Erich Larsen of the Dow Chemical Company.

distilled water and baked overnight at 150 C to evaporate any traces of methoxyflurane and to achieve a constant weight. A portion was converted to the Hyamine salt[9] (study of Subject 1) or suspended as a finely ground precipitate with the aid of a thixotropic gel of finely divided silica (Cab-O-Sil)[10] (studies of Subjects 2, 3, 4, and 17) and added to 15 ml of toluene containing 4 gm of PPO (2,5 diphenyloxazole) and 50 mg of POPOP (2-S-phenyloxazolyl benzene) per liter (Liquifluor, T.M. Pilot Chemicals, Inc.). These were counted on a Tricarb scintillation counter. The efficiency of counting a standard, labeled $Ba^{14}CO_3$ as a Hyamine salt was 70.2 per cent and that of counting a $Ba^{14}CO_3$ suspension in Cab-O-Sil, 55.8 per cent. The overall reproducibility of transfer and counting was ±0.54 per cent (SD) for Hyamine salts having a specific activity of 75 dpm/mg, and it was ±0.96 per cent for $Ba^{14}CO_3$ suspensions of similar specific activity; for samples having specific activities in the range of 0.5 to 1 dpm/mg the reproducibility was about ±5 per cent. To establish that no labeled methoxyflurane (specific activity 15.6×10^6 dpm/ml) was entrained with the $BaCO_3$, three samples were revolatilized with 3 N HCl, driven through a scrubbing tower containing unlabeled methoxyflurane, and recovered in CO_2-free 1 N NaOH; recounting indicated recoveries of 77.2, 79.4 and 77.5 per cent, suggesting that little or no ^{14}C-methoxyflurane had contaminated the original samples.

Urinary Fluoride

Urine specimens from seven patients who received methoxyflurane but no other fluorine-containing drugs for elective operations and five patients who received no fluorinated drugs but were anesthetized with thiopental, nitrous oxide and *d*-tubocurarine for elective operations were collected in polyethylene bottles; the volumes and times of collection were noted. Twenty-four-hour urine samples were obtained from the unpremedicated volunteer exposed to ^{14}C-labeled methoxyflurane. In addition to being analyzed for inorganic and total fluorine content, the urine specimens of the volunteer were analyzed for ^{14}C radioactivity. Fractions of each specimen were stored at 4 C in polyethylene for analysis of fluoride ion and in glass vials sealed with aluminum-lined caps for analysis of organic fluorine. Inorganic fluoride concentrations were measured with a Fluoride Ion Activity Electrode (Orion Research, Inc. Model 94-09), with the *p*H of the sample adjusted to between 6.3 and 6.7 with a phosphate buffer and NaOH or HCl and the ionic strength of the sample adjusted to about 0.15 M.[11] All measurements were made against standard solutions of ACS reagent grade, oven-dried NaF in buffered 0.9 per cent saline solution. Readings obtained from a Radiometer *p*H Meter 22, using a saturated calomel reference electrode, varied less than 1 millivolt per day for a given standard solution; the voltage changed 58 ± 1 mv per log concentration change. When deionized distilled water is used for all dilutions, the method can detect 0.2 μg of F^- per ml of sample.

Urinary Total Fluorine

Organic fluorine was converted to inorganic fluoride by combustion in an oxygen–hydrogen flame and collected in 0.1 N NaOH after the method of Sweetser.[12] The quartz Wickbold apparatus was reduced in size to permit convenient evaporation of 1 ml of sample from a quartz boat into a flame of 1 l/min of hydrogen, a slight excess of oxygen, and sufficient vacuum (40 mm Hg) on the collection vessel to maintain the interior of the combustion chamber at a pressure slightly below atmospheric. The collection vessel was initially charged with 3 ml 0.1 N NaOH, and was rinsed with 2 ml 0.1 N NaOH to make a total volume of approximately 15 ml, including the water generated by the flame. Fluoride ion activity was measured, after neutralization with HCl and adjustment of ionic strength, with the fluoride ion activity electrode. This method is sensitive to 2 micrograms of fluorine per ml of sample.

Identification of Urinary Fluorine Hydrocarbons

Urine specimens collected from additional patients during the first three days following exposure to methoxyflurane, and containing quantities of organic fluorine between 93 and 213 mg/l, were adjusted to *p*H 2 with H_2SO_4

TABLE 1.

Subject	Sex	Age (years)	Weight (kg)	Diagnosis	Physical Status	Operation	Anesthetics	Anesthesia Time (min)	Duration of Exposure to Methoxyflurane (min)	Average Concentration of Methoxyflurane (per cent)	Observations	Est'd Uptake ml (liquid)
1	M	66	64.5	Inguinal hernia	I	Herniorrhaphy	N_2O, halothane, thiamylal ^{14}C-methoxy flurane, succinylcholine	125	31	0.8	^{14}C-methoxy-flurane uptake, excretion; $^{14}CO_2$ excretion	3.2 (2)*
2	F	52	62	Sarcoma, chest wall, recurrent	II	Excision, skin graft	Same as above	225	67	0.8	$^{14}CO_2$ excretion	0.4 (3)*
3	M	23	73	Inguinal hernia	I	Herniorrhaphy	Same as above	90	35	0.75	$^{14}CO_2$ excretion	
4	M	61	75	Postoperative aortic prosthesis	III	Replacement of prosthesis	Same as above, plus carbocaine epidural	525	60	0.41	^{14}C-methoxy-flurane uptake; $^{14}CO_2$ excretion	5.05 (7)*
5	F	47	68	Acoustic neuroma	II	Craniotomy	N_2O, thiopental, methoxyflurane succinylcholine	615	577	0.45	Urinary F^-	
6	M	60	105	Meningioma	II	Craniotomy	Same as above	450	450	0.52	Urinary F^-	
7	M	53	72	Carcinoma of colon	I	Resection of colon	Same as above	305	305	0.37	Urinary F^- total F	
8	F	37	72	Cerebro-vascular accident	IV	Tendon transplant	Same as above	120	55	0.90	Urinary F^-; total F	
9	M	60	58	Carcinoma of esophagus	I	Thoracolap-arotomy	Same as above	210	105	0.34	Urinary F^-; total F	
10	F			Carcinoma of pancreas	III	Exploratory laparotomy	Same as above	180	90	0.38	Urinary F^-; total F	
11	F	73	63	Meningioma	III	Craniotomy	Same as above	315	234	0.48	Urinary F^-; total F	

TABLE 1. (*Continued*)

Subject	Sex	Age (years)	Weight (kg)	Diagnosis	Physical Status	Operation	Anesthetics	Anesthesia Time (min)	Duration of Exposure to Methoxyflurane (min)	Average Concentration of Methoxyfluorane (per cent)	Observations	Est'd Uptake ml (liquid)
12	F	72	80	Duodenal ulcer, cholelithiasis	II	Pyloroplasty, cholecystectomy	Thiopental, N_2O, succinylcholine, α-tubocurarine	155	0	0	Urinary F^-	
13	F	40	53	Carcinoma of pancreas	I	Exploratory laparatomy	Same as above	155	0	0	Urinary F^-	
14	F	60	62	Carcinoma of esophagus	II	Esophagectomy	Thiopental, N_2O, α-tubocurarine	290	0	0	Urinary F^-	
15	F	54	83	Carcinoma of tongue	I	Mandibulectomy, radical neck dissection	Thiopental, N_2O, *d*-tubocurarine, nasolysen	520	0	0	Urinary F^-; total F	
16	F	60	57	Metastatic carcinoma	III	Adrenalectomy	Thiopental N_2O, *d*-tubocurarine	205	0	0	Urinary F^-; total F	
17	M	51	82	Healthy volunteer	I		^{14}C-methoxyflurane	120	120	"Constant alveolar concentration"	^{14}C-methoxyflurane uptake; $^{14}CO_2$, urinary ^{14}C, F^-, total F	9.0 ml (closed system)

* Number of simultaneous measurements of inspired and expired air concentrations of methoxyflurane and ventilation rate upon which the estimate of uptake was based.

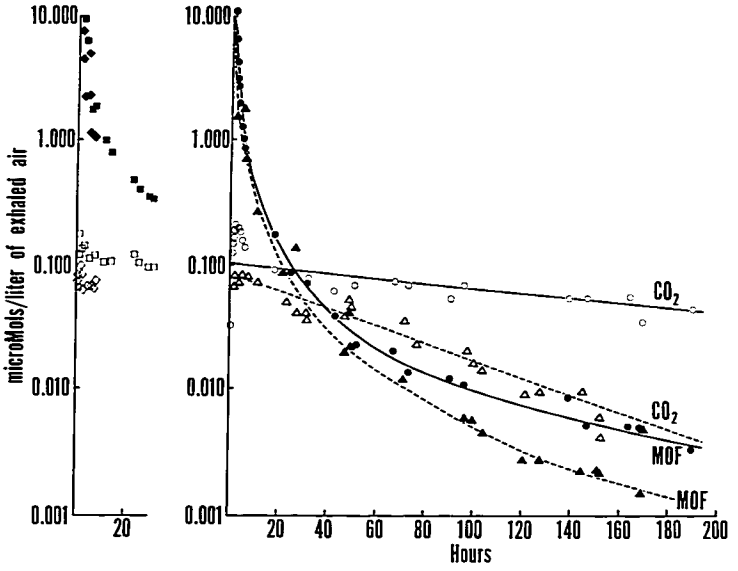


FIG. 1. Concentrations of methoxyflurane (MOF) and $^{14}CO_2$ in exhaled air of subjects 1–4. Subject (diamonds) and Subject (squares) were studied for seven and 30 hours, respectively, and their values are shown on the left to illustrate the consistency of the responses. Subject 3 (triangles and dotted lines) and Subject 4 (circles and solid lines) were studied for 168 and 188 hours, respectively. See text for details of responses. Time is measured from the beginning of exposure to methoxyflurane.

and extracted by vigorous shaking with petroleum ether–butanol, equal parts. The organic phase was separated and shaken with water maintained at a *p*H between 7 and 10 by addition of dilute NaOH. The aqueous phase, which contained 50 to 70 per cent of the organic fluorine of the urine, was concentrated in a flash evaporator, placed on a Dowex 1–formate column and eluted with 1 per cent KBr or 1 per cent KCl. Twenty-ml eluent fractions were analyzed for inorganic fluoride, organic fluorine and glucuronide. The three fractions containing the bulk of organic fluorine (10–30 per cent of the original amount) were acidified with H_2SO_4, extracted with petroleum ether–butanol, and returned to water by neutralization with minimal quantities of NaOH or KOH. The water phase was dried by lyophilization. This product was analyzed for fluorine and potassium, and was examined by fluorine and proton nuclear magnetic resonance and by infrared and mass spectrometry.*

Samples of urine and aqueous solutions of fluorine-rich extracts were stored at room temperature and at 4 C. Changes in the quantities of free fluoride ion and total fluorine were followed for several days. There was a progressive loss of organic fluorine with an increase in fluoride ion, the rates of change of which increased with decreasing *p*H and increasing temperature.

* Grateful acknowledgment is made to Ronald Herz, M.D., for the fluorine NMR analyses, to Josef Fried for the IR analyses, and to Wilbert H. Urry, Ph.D., for fluorine and proton NMR and precise mass spectrometry analyses.

Identification of Dichloroacetic Acid

Two liters of urine obtained from a patient on the third day after exposure to methoxyflurane were acidified to *p*H 2 and extracted with ether for 96 hours. The ether extract was shaken with dilute NaOH; the aqueous phase was neutralized and taken to dryness in a flash evaporator at 60 C. The extract was dissolved in butanol, evaporated to 2.5 ml of an oily syrup and diluted to 7 ml with $CHCl_3$. Two ml of the latter solution were placed on a silicic acid–Celite column and eluted with $CHCl_3$. The remainder of the extract was converted to the p-toluidine derivative by the method of Bruening.[12] The melting point of the product was compared with that of a similarly prepared p-toluidine derivative of dichloroacetic acid.

Results

^{14}C-labeled carbon dioxide was present in the first samples of exhaled air obtained after exposure to ^{14}C-methoxyflurane. Five minutes after initial exposure, the concentration of $^{14}CO_2$ exhaled by Subject 4 was 17 per cent of the maximum achieved. Maximum concentrations, averaging 0.16 micromols of $^{14}CO_2$ per liter of exhaled air, were present between one and three hours after initial exposure (fig. 1). The specific activity of carbon dioxide in exhaled air varied irregularly during the first 48 hours, then decreased exponentially, with biological half-lives of 42 hours in Subject 3, 156 hours in Subject 4 (fig. 1), and 35 hours in the healthy volunteer (Subject 17) (fig. 5). The absorbed methoxyflurane converted to carbon dioxide by Subject 4 amounted to 21.6 per cent, or 52.4 per cent, of that which was not exhaled as unaltered methoxyflurane (fig. 3). The healthy volunteer, Subject 17, excreted 7.1 per cent of the absorbed methoxyflurane as $^{15}CO_2$.

The excretion of methoxyflurane in exhaled air declined in a manner suggesting multiple exponents, or compartments (fig. 1). In studies of Subjects 3 and 4, graphic analysis revealed three compartments with biological half-lives of 31 hours, 9 hours, and 37 minutes,

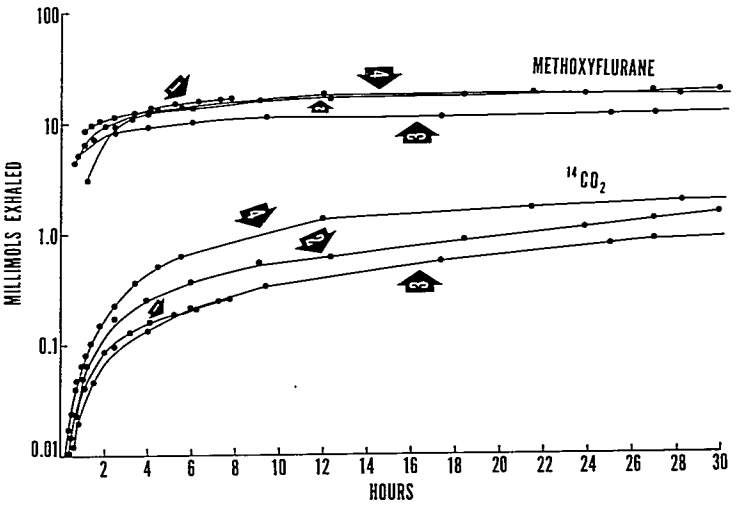


FIG. 2. Accumulative excretion of methoxyflurane (MOF) and $^{14}CO_2$ in Subjects 1–4 during the first 30 hours after the beginning of exposure.

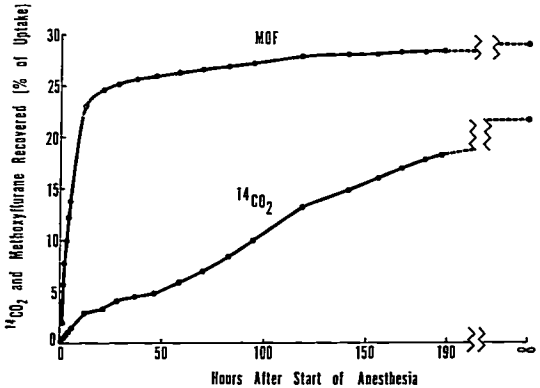


FIG. 3. Accumulative excretion of methoxyflurane (MOF) and $^{14}CO_2$ in Subject 4, expressed as percentage of uptake. Total uptake has been estimated by extrapolation of the excretion curve (fig. 1) after 48 hours.

and 60 hours, 6 hours, and 40 minutes, respectively. Approximately 50 per cent of the methoxyflurane ultimately exhaled unaltered was excreted in 4 hours, and by 30 hours an average of 69 per cent had been excreted (figs. 2, 3, and 6). The total quantity of methoxyflurane exhaled by Subject 4 in unaltered form and as labeled carbon dioxide was estimated to be 50.6 per cent of the amount absorbed, of which 29 per cent was unaltered methoxyflurane and 21.6 per cent was $^{14}CO_2$ (fig. 3).

The quantities of methoxyflurane absorbed by the patients whose urines were examined for excretion of fluorine (table 1) were not

TABLE 2. Excretion of Free Fluoride Ion

Anesthesia	Subject	Exposure (min)	Rate of Excretion			Biological Half-life (hours)	Total Recovered† (mg)	Hours of Collection
			Preanesthetic (μg/min)	Peak (μg/min)	Average* (μg/min)			
Methoxyflurane								
	8	55	0.68 (3)‡	65	17 (10)	47	161	210
	10	90	0.80 (3)	41	28 (7)	40	115	217
	9	165	0.41 (1)	34	30 (7)	54	243	263
	11	234	—	76	42 (14)	57	154	85
	7	305	1.37 (1)	120	58 (15)	77	293	85
	6	445	1.00 (1)	89	68 (11)	86	320	80
	5	577	—	275	75 (11)	—	154	34
Thiopental–N_2O–relaxant								
	13	155	0.11 (1)	1.8	0.65 (14)	—	2.3	51
	12	155	0.75 (6)	1.2	0.77 (11)	—	3.1	66
	14	290	0.42 (4)	0.5	0.29 (6)	—	1.6	89
	16	295	0.51 (4)	1.1	0.52 (8)	—	3.2	96
	15	520	0.77 (5)	1.6	0.29 (1)	—	1.0	37

* Averaged over the initial 80–97 hours, except in the study of subject 5, in which collections over 34 hours were averaged.

† Estimated for total hours of collection only. Quantities excreted during periods in which no collection was made were estimated by interpolation and included.

‡ Numbers in parentheses are numbers of samples.

TABLE 3. Excretion of Total Fluorine

Anesthesia	Subject	Exposure (min)	Rate of Excretion			Biological Half-life (hours)	Total Recovered† (mg)	Hours of Collection
			Preanesthetic (μg/min)	Peak (μg/min)	Average* (μg/min)			
Methoxyflurane								
	8	55	1.6 (1)‡	316	103 (7)	34	943	210
	10	90	9.3 (1)	213	176 (9)	33	690	217
	9	165	—	204	145 (7)	39	1,040	283
	11	234	—	425	120 (12)	—	—	20
	7	305	—	787	330 (13)	46	1,710	85
Thiopental-N_2O-relaxant								
	16	295	3.2 (2)	6.4	1.9 (6)	—	18.7	96
	15	520	3.3 (1)	30.4	4.4 (6)	—	16.0	37

Numbers in parentheses are number of samples. Dashes indicate values which were not determined.
* Averaged over the initial 80–97 hours, except in the study of subject 11, in which collections in the first 20 hours were averaged.
† Estimated for total hours of collection only. Quantities excreted during periods in which no collection was made were estimated by interpolation.

measured, but can be assumed to have increased as duration of exposure increased. Urinary excretion of free fluoride ion rose from an average preanesthetic rate of 0.69 μg/min to 17–75 μg/min, averaged over the first three to four days following exposure to methoxyflurane (table 2). Peak rates of excretion occurred between two and 44 hours (average 17 hours) and ranged from 34 to 275 μg/min. There was no sustained elevation of excretion of free fluoride ion in patients anesthetized with thiopental, nitrous oxide and relaxant. Average and peak rates of excretion tended to increase with the duration of exposure to methoxyflurane. Total quantities of fluoride ion excreted during the periods of study ranged

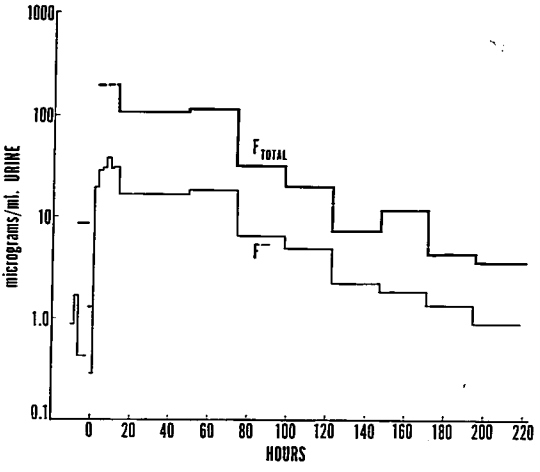


FIG. 4. Concentrations of inorganic fluoride and total fluorines in the urine of Subject 10. Discontinuities represent periods in which no collection was made. Estimated total excretions are based on interpolations of missed periods (see text and tables 2 and 3).

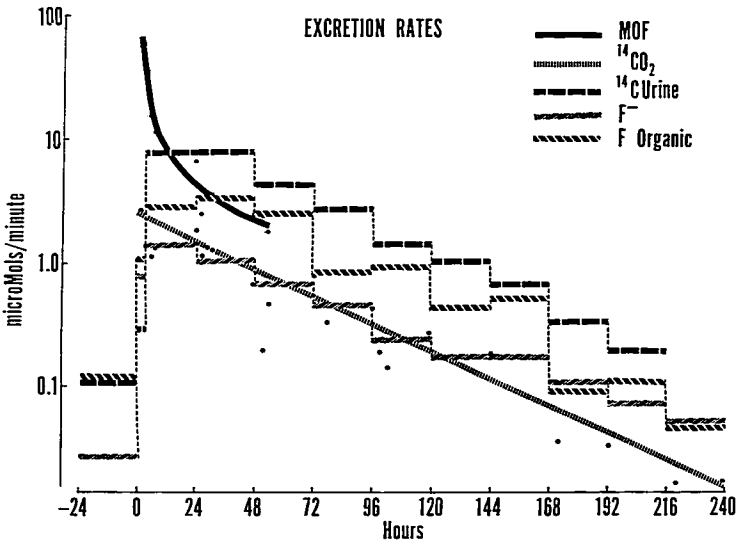


FIG. 5. Excretion of methoxyflurane and metabolites in Subject 17, the healthy volunteer.

from 115 to 320 mg and tended to increase with the duration of exposure.

Excretion of total fluorine (inorganic plus organic) increased from an average preanesthetic rate of 4 μg/min to average between 103 and 330 μg/min, and tended to depend on the length of exposure to methoxyflurane (table 3). In patients who did not receive fluorinated drugs during anesthesia output of total fluorine did not increase, except transiently. The excretion of total fluorine rose to peak values of 213 to 787 μg/min within two to 13 hours following initial exposure and then declined approximately exponentially (fig. 4), with biological half-lives which ranged from 33 to 49 hours and seemed to depend on duration of exposure to methoxyflurane. Total quantities of fluorine ranging from 690 to 1,710 mg were excreted in 85 to 263 hours of collection. These quantities are equivalent to 18.2 to 45 millimols of methoxyflurane, or 2.1 to 5.2 ml of liquid methoxyflurane. The quantities of organic bound fluorine excreted during the periods of collection were equivalent to 15.1 to 37.3 millimols, or 1.75 to 4.3 ml of liquid methoxyflurane.

The volunteer absorbed 9.91 ml (82.5 mMol) of liquid methoxyflurane. He is estimated to have excreted 3.4 ml (29.4 mMol, or 35.4 per cent) in unaltered form, assuming a 35-hour biological half-life after 48 hours (figs. 5 and 6). In ten days he excreted 6.0 mMols, or 7.1 per cent of the retained quantity, as $^{14}CO_2$, and 34.1 mMol, or 40.0 per cent, as urinary ^{14}C-labeled metabolites. This accounts for 82.5 per cent of the absorbed ^{14}C, of which 47.1 per cent was metabolized. Of the absorbed fluorine, 20.8 mMols, or 24.4 per cent, appeared in the urine; of this, 5.7 mMols, or 6.7 per cent, was fluoride ion, and 15.1 mMols, or 17.7 per cent, was organic fluorine.

IDENTIFICATION OF URINARY FLUORINE HYDROCARBONS

Upon passage through Dowex-I ion-exchange resin, concentrated petroleum ether–butanol

extracts of urine exhibited a single broad peak of organic fluorine and a smaller peak of a glucuronide which coincided with the early portion of the organic fluorine peak (fig. 7). Similarly treated extracts of the volunteer's urine revealed that practically all of the ^{14}C-labeled urinary metabolites were eluted in the same fractions as the organic fluorine (fig. 8).

The three fractions containing the bulk of organic fluorine were acidified, re-extracted with petroleum ether–butanol and returned to water with a minimum amount of KOH. The water was removed by lyophilization. The product, a white precipitate which on storage slowly turned to a buff-colored sticky material, contained 10 per cent fluorine and 12 per cent potassium. A strong peak at 1,650 cm^{-1} in the infrared spectrophotogram indicated the presence of a carboxyl group. Fluorine nuclear magnetic resonance scans disclosed a single, strong peak, without splitting, at 4,641 cps establishing the absence of hydrogens on carbons adjacent to the C—F group. Hydrogen nuclear magnetic resonance revealed a single, strong peak consistent with a methoxy group. Mass spectroscopy of the salt showed major peaks at mass numbers 81 and 44. Precise mass spectroscopy showed the first peak to have mass of 81.0452, which is consistent only with a fragment of the structure $C_2H_3OF_2$ (theoretical mass 81.046). Precise mass spectroscopy established the second fragment of mass 44.000 to be CO_2. These observations strongly support the conclusion that the principal urinary organic fluorine compound has the structure CH_3OCF_2COONa.

An ether extract, placed on silicic acid–celite columns and eluted with chloroform, yielded a strong peak of acid at the same eluent volume as dichloroacetic acid, equivalent to 178 mg of dichloroacetic acid per 2 liters of urine. The p-toluidine derivative of the extract melted at 149–154 C. A similarly prepared p-toluidine derivative of dichloroacetic acid melted at 154–155 C.

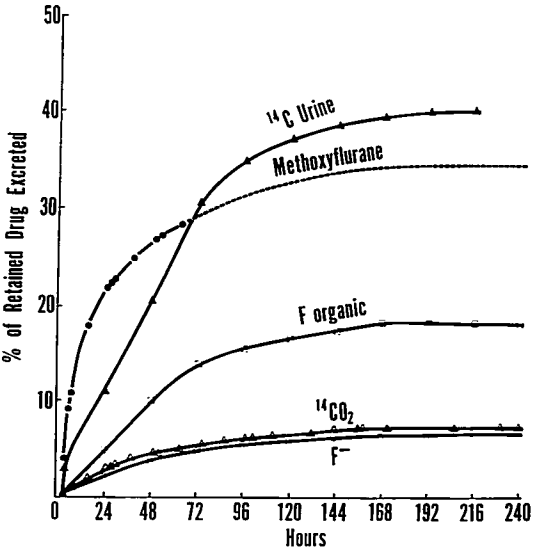


FIG. 6. Accumulative excretion of methoxyflurane and its metabolites in Subject 17. The dashed line, representing methoxyflurane excreted beyond 48 hours, is the half-life of 35 hours. Time is measured from the beginning of exposure.

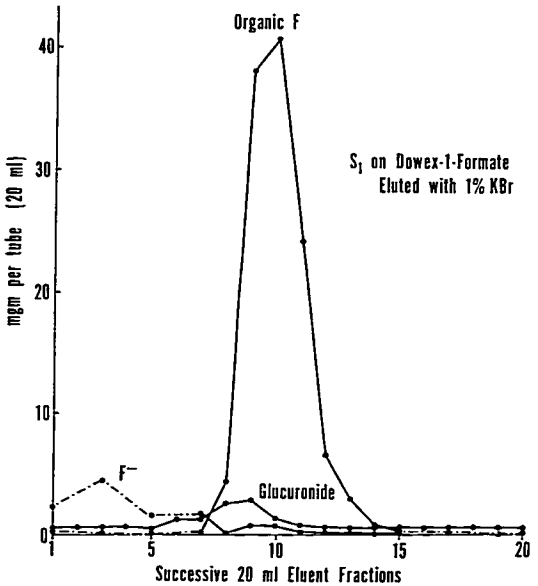


FIG. 7. Distribution of organic fluorine, glucuronide and fluoride ion in eluent fractions following passage of a petroleum ether–butanol extract of acidified urine through Dowex 1-formate (Subject 11).

Discussion

The data presented in figures 3 and 6 indicate that man may exhale, unaltered, less than half of the methoxyflurane absorbed during an hour of anesthesia. A portion of the remainder (as much as 21 per cent) is converted to CO_2 derived from the methoxy group. A larger fraction of the methoxy-carbon is excreted in the urine as nonvolatile material. The ratio of ^{14}C-labeled urinary metabolites to $^{14}CO_2$ observed in rats by Van Dyke and associates averaged 3.3.[1, 14] The results of the present studies are consistent with a similar ratio in man.

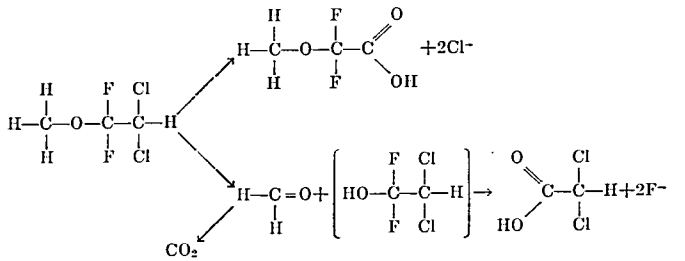
Significant quantities of nonvolatile fluorine are excreted in urine following exposure to methoxyflurane. A rather constant one-sixth appears as fluoride ion (tables 2 and 3). The release of fluoride ion *in vivo* probably was secondary to hydrolysis of the ether linkage. Van Dyke[4] has shown in the rat that cleavage of the ether is enzymatic. Hudlicky[15] reports that spontaneous hydrolysis of C—F bonds proceeds easily in terminal —CF_2— groups when oxygen is bonded to the same carbon. The identification of significant quantities of dichloroacetic acid in the urine of patients exposed to methoxyflurane supports this mechanism.

The organic fluorine found in the urine was identified as being mainly in one form, the salt of CH_3—O—CF_2—COOH, the source of which has to be the dechlorination of methoxyflurane. The quantities of organic fluorine excreted during the periods of study ranged from 575 to 1,417 mg, equivalent to 15.1 to 37.3 millimols of methoxyflurane. Following 55 minutes of anesthesia, one patient (Subject 8) excreted the equivalent of 20.1 mM, or 2.4 ml (liquid) of methoxyflurane as nonvolatile organic fluorine. The fractions from the ion-exchange columns which con-

tained the bulk of organic fluorine were accompanied by much smaller quantities of glucuronide.

The foregoing data substantiate the routes of biotransformation proposed by Van Dyke and Chenoweth,[16] but fail to support the contention that the metabolites are excreted as glucuronide conjugates, and suggest that oxidations proceed to carboxyl formation.

The reactions appear to be:



The dechlorination proceeds at a greater rate than the cleavage of the ether linkage, as it does in the rat.[1]

If simultaneous or sequential dechlorination and ether cleavage occur in the same molecule, oxalic acid could be another product of the above reactions. Oxalate was not sought in the present study, but has been identified within renal tubules post mortem.[17, 18]

The time courses of the elimination of the various metabolites can shed some light on the dynamics of the enzymatic degradation processes. Carbon dioxide derived from the methoxy group appeared in exhaled air within six

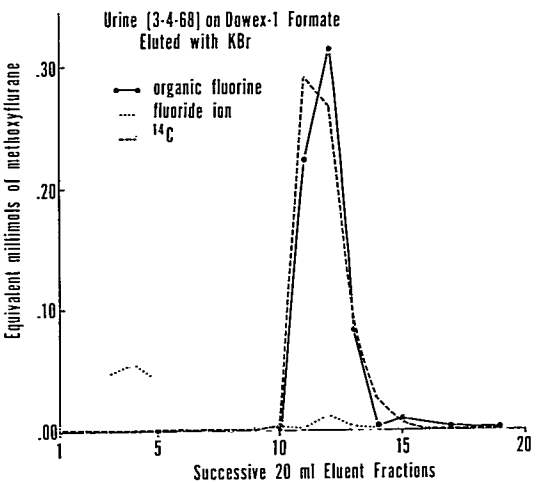


FIG. 8. Distribution of organic fluorine, fluorine ion and ^{14}C-labeled urinary metabolites in eluent fractions following passage of a petroleum ether–butanol extract of acidified urine of Subject 17, day 2, through an ion-exchange resin column. Quantities are expressed as equivalent millimoles of methoxyflurane.

minutes of initial exposure, and peak excretion rates occurred in one to three hours, or within one-half to two and a half hours after achievement of peak blood concentrations. The biological half-lives of the labeled carbon in two patients were 42 and 156 hours, and in the unpremedicated volunteer the half-life was 35 hours. Assuming that the fate of the methoxy group is similar to that of methanol and formaldehyde, the carbon of which is available for incorporation into carbohydrates and amino acids via the one-carbon pool,[19] the prolonged half-life of the labeled CO_2 is not inconsistent with early and rapid splitting of the ether linkage.

The fate of absorbed inorganic fluoride is well known, and its excretion by the kidney is prompt. Man deposits in bone 35 to 50 per cent of the fluoride ion ingested in quantities above the average daily intake. Half of the remainder appears in the urine in three to four hours, and the urinary excretion rate returns to control levels in 24 hours.[20] A part of that absorbed into bone is released into the urine over several weeks, and another portion is released much more slowly, about half of that remaining being lost in two years.[21] In the present studies, excess inorganic fluoride began to appear in the urine between two and four and a half hours after the initial exposure, reached maximum excretion rates in two to 44 hours (average 17 hours), and declined approximately exponentially, with a half-life of 36 to 46 hours. These facts support the conclusion that, although blood levels of methoxyflurane consistently fall to 10 per cent of peak concentrations in four hours and to 1 per cent in 24 hours following cessation of anesthesia, there is continued transfer of methoxyflurane from storage depots to sites of enzymatic attack in quantities which permit maximum rates of hydrolysis for as long as 40 hours.

The time course of dehalogenation should be reflected by the appearance of nonvolatile, organic fluorine and the nonvolatile ^{14}C-labeled urinary metabolites. The latter, although not identified structurally in the present study, should be largely the same compound or compounds as those containing organic fluorine. These appear in larger quantity, but have excretion curves parallel to that of fluoride ion (figs. 4, 5, and 6), tending to confirm the conclusion that the rates of the degradation processes are dependent on and limited by the rate of release of unaltered drug from storage depots. The storage depots appear not to approach depletion before nine to 12 days, depending on the duration of anesthesia and the activity of the subject.

Maximum excretion rates of the various metabolites are delayed. Peak excretion rates of labeled CO_2, total fluorine, and fluoride ion occur on the average at two hours, eight hours and 17 hours, respectively. Part of the delay may be due to binding of nonvolatile metabolites in tissues, such as has been demonstrated for chloroform.[22] The delay may also reflect a progressive increase in the capacity of the enzymes of the body to metabolize drugs. Two processes could contribute to the latter. Depression of enzymatic drug detoxication occurs in the presence of high levels of drugs acted upon by these enzymes. Rubin and Lieber[23] have shown that "physiologic" concentrations of alcohol prolong drug degradation. Anesthetic concentrations of methoxyflurane may have a similar effect. Second, an increase in total enzymic activity may occur as a consequence of induction of microsomal enzymes by prolonged exposure to low levels of methoxyflurane, as has been demonstrated by Van Dyke.[14]

A high degree of metabolic degradation of methoxyflurane relative to other volatile anesthetics is predictable on the basis of the more prolonged residence of methoxyflurane in the body. Its blood–air partition coefficient of 12–13 determines that, as it is discharged from tissue storage depots, it will be recirculated several times through the hepatic circulation before being transferred to alveolar gas and exhaled.

The authors thank Dr. Harry J. Lowe and Mr. Karl Hagler for performance of the closed-circuit, constant alveolar concentration anesthesia in study 17. Grateful acknowledgment is made to Mr. Eddie Bowie for his assistance in developing the analytical methods and collecting the samples.

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Drugs

GLUCAGON The effects of glucagon on the coronary arteries of the dog were studied. The addition of glucagon to the perfusate decreased vascular resistance, and increased myocardial oxygen consumption, heart rate and myocardial contractility. When glucagon was added to the perfusate of the arrested heart, no change in the above parameters occurred. The decrease in coronary resistance in the contracting heart after glucagon is secondary to the metabolic effects of the increased myocardial contractility and heart rate, rather than a direct vasodilating effect on coronary vessels. (*Moir, T. W., and Naylor, W. G.: Coronary Vascular Effects of Glucagon in the Isolated Dog Heart, Circ. Res.* 26: 29 (*Jan.*) 1970.)