

Renal Effects and Metabolism of Sevoflurane in Fischer 344 Rats:

An In-vivo and In-vitro Comparison with Methoxyflurane

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Sevoflurane, 1.4 per cent (MAC), was administered to groups of Fischer 344 rats for 10 hours, 4 hours, or 1 hour; additional rats received 0.5 per cent methoxyflurane for 3 hours or 1 hour. Urinary inorganic fluoride excretion of sevoflurane *in vivo* was a third to a fourth that of methoxyflurane. However, using hepatic microsomes, sevoflurane and methoxyflurane were defluorinated *in vitro* at essentially the same rate. The discrepancy between defluorination of sevoflurane and methoxyflurane *in vivo* and *in vitro* was probably due to differences in tissue solubility between the drugs. There were no renal functional or morphologic defects following sevoflurane administration. An unexplained adverse effect was significant weight loss, which occurred following all exposures to sevoflurane. (Key words: Anesthetics, volatile, sevoflurane; Anesthetics, volatile, methoxyflurane; Biotransformation, sevoflurane; Kidney, nephrotoxicity.)

SEVOFLURANE[†], (CH₂F-O-CH(CF₃)₂; fluoro-methyl-1,1,1,3,3,3-hexafluoro-isopropyl ether) is a new, nonflammable, fluorinated inhalation anesthetic agent with the following properties: empirical formula, C₄H₂F₆O; boiling point (760 torr), 58.5 °C; liquid density, 1.505 g/ml; vapor pressure (20 °C), 160 torr; blood-gas partition coefficient, 0.59[‡];

oil-gas partition coefficient (corn oil), 55; and MAC, 2.5 per cent.** As part of the animal experiments carried out before an investigational drug is used in man, the renal effects and biotransformation of sevoflurane *in vivo* and *in vitro* were studied in Fischer 344 rats.

Methods and Materials

IN-VIVO STUDIES

Thirty 12-month-old, male Fischer 344 rats were divided at random into five treatment groups of six rats each, as follows: Group I, no anesthesia; Group II, 0.5 per cent methoxyflurane for 3 hours; Group III, 0.5 per cent methoxyflurane for 1 hour; Group IV, 1.4 per cent sevoflurane†† for 10 hours; Group V, 1.4 per cent sevoflurane for 4 hours. At a later date six additional 12-month-old male Fischer 344 rats received 1.4 per cent sevoflurane for 1 hour. All animals were treated identically except as noted in their group designations. Animals were housed in individual metabolic cages where food and tap water containing inorganic fluoride, 21 mg/kg, and one part per million (52.6 µM), respectively, were allowed *ad libitum*. Artificial light was present from 7 AM to 6 PM each day, and room temperature was maintained at 21–23 °C. Three days were allowed for the animals to adapt to the metabolic cages; during this time a 1-ml tail blood sample was obtained for control blood tests. On each of days 4 through 6, control 24-hour urine collections were obtained. On experiment day 7, sevoflurane was administered to the rats in a plastic chamber of 100-liter volume. In order to conserve drug, a closed system was

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†† MAC in mice was reported as 1.4 vol per cent; this value was utilized in the present study. Subsequently, we determined MAC to be 2.5 vol per cent in Fischer 344 rats.

employed. Soda lime was placed on the floor of the anesthesia chamber to a depth of $\frac{1}{2}$ inch and a plastic wire mesh laid on top of it. After the rats were in the chamber, 10 ml of liquid sevoflurane were injected onto a gauze sponge and vaporized within the chamber. Ambient sevoflurane concentration was measured by gas chromatography, and 2-5-ml increments of drug were vaporized approximately every 15 minutes in order to maintain a concentration of 1.4 per cent. Oxygen was added to the chamber, intermittently, in order to maintain an atmospheric concentration of 50 per cent. Methoxyflurane was administered with a Pentec vaporizer employing a semiclosed system; oxygen flowing at 6 l/min was the carrier gas.

Anesthetic concentrations in the cage were determined with a Varian No. 1440 gas chromatograph and oxygen concentration with an Instrumentation Laboratories No. 402 oxygen analyzer. Rectal temperature was continuously monitored with a Yellow Springs multichannel telethermometer and was maintained between 37 and 39°C with the aid of a water mattress on the floor of the chamber. During the last 30 minutes of anesthesia, a 0.3-ml blood specimen was obtained from the tail of each animal in order to measure anesthetic concentration, pH, P_{O_2} , and P_{CO_2} . After anesthesia, rats received 100 per cent oxygen until awake and then were returned to their individual metabolic cages, where 24-hour urine collections were continued for the next four days. One-milliliter tail blood samples were obtained on postanesthesia days 1 and 2; feces were collected on day 1. Two rats from each group were sacrificed at the end of experiment day 7 (postanesthesia day 1), and the remaining animals were sacrificed at the end of postanesthesia day 4. At the time of sacrifice, blood was obtained for biochemical analysis, and kidney tissue was removed for study by light and electron microscopy. Serum and urine specimens were analyzed for sodium and potassium (Instrumentation Laboratories No. 143 Flame Photometer), urea nitrogen (Technicon AutoAnalyzer), fluoride activity (Orion ion-specific fluoride electrode and No. 801 Ionalyzer), and osmolality (Fiske Model 130 Osmometer). Urinary organic fluoride was determined employing a combustion

method. Laboratory methods have been reported.²⁻³ Fecal inorganic fluoride activity was determined by placing samples in 100-ml flasks containing 50 ml of 2.5 M sodium acetate buffer, pH 4.8. Each sample was then homogenized and analyzed for fluoride ion activity. Sodium, potassium, osmolal, inorganic and organic fluoride excretions were calculated, as was urea nitrogen clearance. Rats were weighed daily.

PEAK SERUM FLUORIDE

Six additional Fischer 344 rats were anesthetized with 1.4 per cent sevoflurane for 4 hours. Tail blood was obtained 1, 4, 12, 24, and 48 hours after anesthesia and serum inorganic fluoride activity was determined.

IN-VITRO STUDIES

Hepatic microsomes were prepared from seven adult male Fischer 344 rats, using standard methods.^{4,5} In the final reaction mixture, 5 mg of microsomal protein were incubated with 1 mM sevoflurane or methoxyflurane in the presence of oxygen and a NADPH-generating system. The reaction was stopped after 30 minutes and the mixture was vacuum-evaporated to dryness. The residue was resuspended in acetate buffer and inorganic fluoride concentration determined.

STATISTICAL ANALYSIS

The mean value of each variable for each rat for the three-day preanesthetic period was calculated and means were determined for each group. Daily means of postanesthetic data were computed for each group and compared with the preanesthetic mean. The effects of increasing methoxyflurane and sevoflurane dosages were determined by subtracting the preanesthetic mean from the mean of determinations for each postanesthetic day. These values were compared with those of unanesthetized control rats, Group I, for the same periods. Student's *t* test was used for statistical analysis.

Results

IN-VIVO STUDIES

Prior to anesthesia all variables for all groups were within normal limits. There

TABLE 1. Serum and Urinary Values, Mean \pm SE

| | Period | Serum | | | | Urine | | | | |
|---------------------------------------------------|-----------------|-------------------|-------------------------|---------------------------------|--------------------------------------|-------------------|----------------------|-------------------------|---------------------------------|--------------------------------------|
| | | Sodium (mEq/l) | Osmolality (mOsm/kg) | Urea Nitrogen (mg/100 ml) | Inorganic Phosphate (μ M) | Sodium (mEq/l) | Potassium (mEq/l) | Osmolality (mOsm/kg) | Urea Nitrogen (mg/100 ml) | Inorganic Phosphate (μ M) |
| Group I, control | Preanesthetic* | 138 \pm 0.3 | 294 \pm 1 | 18.0 \pm 0.5 | 2.8 \pm 0.1 | 135 \pm 6 | 332 \pm 9 | 2,233 \pm 48 | 4,817 \pm 116 | 485 \pm 12 |
| | Postanesthetic* | 139 \pm 0.2 | 295 \pm 1 | 19.1 \pm 0.8 | 2.9 \pm 0.1 | 157 \pm 6 | 375 \pm 10 | 2,385 \pm 68 | 5,094 \pm 198 | 588 \pm 68 |
| Group II, 0.5 per cent Methoxyflurane, 3 hours | Preanesthetic | 140 \pm 0.1 | 297 \pm 1 | 18.2 \pm 0.3 | 2.6 \pm 0.1 | 139 \pm 6 | 324 \pm 14 | 2,091 \pm 59 | 4,397 \pm 166 | 483 \pm 23 |
| | Postanesthetic | 140 \pm 0.2 | 304 \pm 3 | 19.3 \pm 0.9 | 76.3 \pm 8.0 | 100 \pm 4 | 184 \pm 14 | 1,411 \pm 97 | 2,876 \pm 211 | 5,478 \pm 194 |
| Group III, 0.5 per cent Methoxyflurane, 1 hour | Preanesthetic | 139 \pm 0.2 | 296 \pm 0 | 19.3 \pm 0.3 | 2.7 \pm 0.1 | 139 \pm 4 | 324 \pm 7 | 2,202 \pm 46 | 4,653 \pm 151 | 468 \pm 8 |
| | Postanesthetic | 141 \pm 0.4 | 298 \pm 0 | 20.6 \pm 0.2 | 40.0 \pm 3.9 | 123 \pm 6 | 298 \pm 18 | 2,106 \pm 101 | 4,348 \pm 233 | 5,109 \pm 170 |
| Group IV, 1.4 per cent Sevoflurane, 10 hours | Preanesthetic | 140 \pm 0.3 | 298 \pm 1 | 19.0 \pm 0.3 | 2.4 \pm 0.1 | 149 \pm 4 | 349 \pm 9 | 2,318 \pm 37 | 4,846 \pm 105 | 513 \pm 14 |
| | Postanesthetic | 141 \pm 0.8 | 303 \pm 2 | 19.0 \pm 0.8 | 13.8 \pm 1.1 | 99 \pm 23 | 292 \pm 27 | 1,914 \pm 129 | 3,897 \pm 298 | 7,838 \pm 589 |
| Group V, 1.4 per cent Sevoflurane, 4 hours | Preanesthetic | 139 \pm 0.3 | 295 \pm 0 | 19.7 \pm 0.4 | 2.7 \pm 0.1 | 144 \pm 6 | 333 \pm 13 | 2,064 \pm 59 | 4,390 \pm 120 | 476 \pm 25 |
| | Postanesthetic | 140 \pm 0.5 | 298 \pm 1 | 18.0 \pm 0.7 | 11.5 \pm 1.8 | 116 \pm 10 | 275 \pm 27 | 1,924 \pm 121 | 3,842 \pm 289 | 4,576 \pm 536 |
| Group VI, 1.4 per cent Sevoflurane, 1 hour | Preanesthetic | 144 \pm 0.2 | 295 \pm 1 | 17.4 \pm 0.3 | 2.3 \pm 0.1 | 123 \pm 6 | 386 \pm 11 | 2,337 \pm 48 | 5,060 \pm 135 | 395 \pm 14 |
| | Postanesthetic | 143 \pm 0.4 | 294 \pm 2 | 18.1 \pm 0.7 | 4.3 \pm 0.3 | 110 \pm 14 | 325 \pm 19 | 2,130 \pm 122 | 4,477 \pm 301 | 2,790 \pm 188 |

* Rats in this group were not anesthetized. Collection periods parallel those for anesthetized rats.

† $P < 0.05$, preanesthetic (days 3-6) vs. postanesthetic (day 7).‡ $P < 0.01$, preanesthetic (days 3-6) vs. postanesthetic (day 7).§ $P < 0.05$, postanesthetic (day 7) vs. postanesthetic, control group (day 7).|| $P < 0.01$, postanesthetic (day 7) vs. postanesthetic, control group (day 7).

TABLE 2. Urinary Volume, Solute Excretion, and Miscellaneous Tests, Mean \pm SE

| Group | Period | Solute Excretion | | | | | Miscellaneous | | |
|---------------------------------------------------|-----------------|----------------------------|---------------------------------|------------------------------------|-----------------------------------|--------------------------------------------------|------------------------------------------------|--------------------------------|----------------------------------------------|
| | | Urine Volume (ml/24 hr) | Sodium Excretion (mEq/24 hr) | Potassium Excretion (mEq/24 hr) | Osmolal Excretion (mOsm/24 hr) | Inorganic Fluoride Excretion (μ M/24 hr) | Organic Fluoride Excretion (μ M/24 hr) | Urea Clearance (ml/min) | Fecal Inorganic Fluoride (μ M/24 hr) |
| Group I, control | Preanesthetic* | 8.9 \pm 0.3 | 1.2 \pm 0.1 | 2.9 \pm 0.1 | 19.7 \pm 0.6 | 4.2 \pm 0.1 | 10.5 \pm 1.4 | 1.7 \pm 0.1 | — |
| | Postanesthetic* | 8.3 \pm 0.3 | 1.3 \pm 0.1 | 3.1 \pm 0.1 | 19.7 \pm 0.5 | 4.9 \pm 0.2 | 5.5 \pm 0.2 | 1.5 \pm 0.1 | 0.8 \pm 0.0 |
| Group II, 0.5 per cent Methoxyflurane, 3 hours | Preanesthetic | 10.9 \pm 0.6 | 1.5 \pm 0.1 | 3.4 \pm 0.1 | 22.3 \pm 0.8 | 5.1 \pm 0.2 | 7.8 \pm 0.7 | 1.8 \pm 0.1 | — |
| | Postanesthetic | 16.94 \pm 1.1 | 1.7 \pm 0.1 | 3.1 \pm 0.2 | 23.5 \pm 1.1 | 9.19 \pm 3.5 | 417.81 [†] \pm 54.6 | 1.7 \pm 0.1 | 1.4 \pm 0.1 |
| Group III, 0.5 per cent Methoxyflurane, 1 hour | Preanesthetic | 10.9 \pm 0.4 | 1.5 \pm 0.1 | 3.5 \pm 0.1 | 23.9 \pm 0.9 | 5.1 \pm 0.2 | 8.4 \pm 0.8 | 1.8 \pm 0.1 | — |
| | Postanesthetic | 11.5 \pm 0.5 | 1.4 \pm 0.1 | 3.4 \pm 0.2 | 24.1 \pm 1.1 | 58.84 [†] \pm 3.6 | 250.71 [†] \pm 11.8 | 1.7 \pm 0.1 | 1.2 \pm 0.1 |
| Group IV, 1.4 per cent Sevoflurane, 10 hours | Preanesthetic | 9.4 \pm 0.5 | 1.4 \pm 0.1 | 3.2 \pm 0.1 | 21.5 \pm 0.9 | 4.8 \pm 0.2 | 9.0 \pm 1.7 | 1.6 \pm 0.1 | — |
| | Postanesthetic | 5.01 \pm 0.5 | 0.64 \pm 0.2 | 1.41 [†] \pm 0.2 | 9.41 [†] \pm 0.8 | 38.24 [†] \pm 3.2 | 142.91 [†] \pm 34.0 | 0.81 [†] \pm 0.1 | 0.9 \pm 0.1 |
| Group V, 1.4 per cent Sevoflurane, 4 hours | Preanesthetic | 10.3 \pm 0.6 | 1.5 \pm 0.1 | 3.4 \pm 0.1 | 20.9 \pm 0.9 | 4.7 \pm 0.1 | 8.3 \pm 1.0 | 1.6 \pm 0.1 | — |
| | Postanesthetic | 9.8 \pm 1.4 | 1.1 \pm 0.1 | 2.5 \pm 0.2 | 18.1 \pm 1.6 | 41.41 [†] \pm 3.0 | 193.61 [†] \pm 17.6 | 1.4 \pm 0.1 | 0.7 \pm 0.1 |
| Group VI, 1.4 per cent Sevoflurane, 1 hour | Preanesthetic | 7.3 \pm 0.2 | 0.9 \pm 0.1 | 2.8 \pm 0.1 | 17.0 \pm 0.4 | 2.9 \pm 0.1 | 2.3 \pm 0.4 | 1.5 \pm 0.1 | — |
| | Postanesthetic | 8.2 \pm 0.6 | 0.9 \pm 0.1 | 2.7 \pm 0.2 | 17.6 \pm 1.5 | 23.24 [†] \pm 2.4 | 117.54 [†] \pm 10.5 | 1.4 \pm 0.1 | 0.7 \pm 0.0 |

* Rats in this group were not anesthetized. Collection periods paralleled those for anesthetized rats.

† $P < 0.05$, preanesthetic (days 3-6) vs. postanesthetic (day 7).‡ $P < 0.01$, preanesthetic (days 3-6) vs. postanesthetic (day 7).§ $P < 0.05$, postanesthetic (day 7) vs. postanesthetic, control group (day 7).|| $P < 0.01$, postanesthetic (day 7) vs. postanesthetic, control group (day 7).

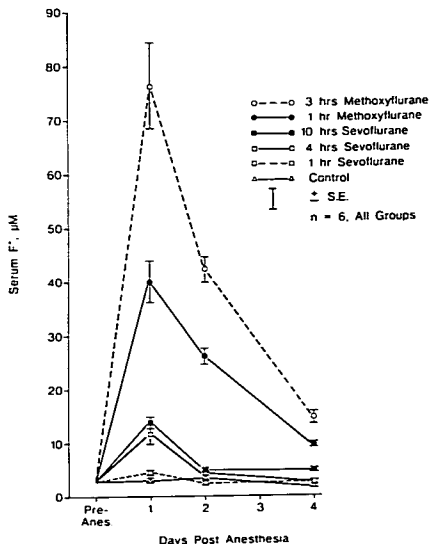


FIG. 1. Daily serum inorganic fluoride concentration after anesthesia. There were dose-related increases following administration of both drugs, with values after methoxyflurane considerably higher than after sevoflurane. Inorganic fluoride concentrations were still elevated four days after methoxyflurane anesthesia; by contrast, they had returned to normal 48 hours after sevoflurane administration.

were no significant differences among groups or among control animals except those that could be attributed to chance (tables 1 and 2). Rats anesthetized with methoxyflurane for 3 hours, Group II, developed slight nephrotoxicity. There was an increase in 24-hour urine output and a reciprocal decrease in urinary osmolality, but no change in BUN. Twenty-four hours after anesthesia, serum inorganic fluoride level was $76.3 \pm 8.0 \mu\text{M}$, a value consistent with slight nephrotoxicity (fig. 1).² Mitochondrial swelling of proximal convoluted tubule cells was visible with electron microscopy. Rats anesthetized with methoxyflurane for one hour showed no abnormality. There also were dose-related increases in urinary inorganic and organic fluoride excretion (fig. 2, table 2).

Sevoflurane administration did not result in a renal defect. After one or four hours of anesthesia, there was no change in variables related to renal function. After 10 hours there was a significant decrease in urinary output,

which probably was secondary to decreased solute excretion. However, BUN was unchanged in all groups, and no renal abnormality was seen with light or electron microscopy. Sevoflurane was defluorinated about a third to a fourth as much as methoxyflurane; there were dose-related increases in mean serum inorganic fluoride concentration and urinary inorganic and organic fluoride excretion. Fluoride levels returned towards preanesthetic values more quickly after sevoflurane than after methoxyflurane administration (fig. 1).

Of potential importance was the substantial weight loss observed in rats anesthetized with sevoflurane. Rats anesthetized for one hour lost 13 grams by the fourth day of the experiment; those anesthetized for 4 hours, 34 grams; those anesthetized for 10 hours, 38 grams. The latter two values are significantly greater than the weight loss which followed 3 hours of methoxyflurane (17 grams) or after no anesthesia (3 grams).

PEAK SERUM FLUORIDE

Mean peak serum inorganic fluoride level following 4 hours of sevoflurane administration occurred 4 hours after anesthesia and was $29.1 \pm 2.5 \mu\text{M}$ (fig. 3).

IN-VITRO STUDIES

Sevoflurane and methoxyflurane defluorinase specific activities were 1.69 ± 0.26 and $1.97 \pm 0.47 \text{ nmol F}^-/30 \text{ min/mg protein}$, respectively. These values are not significantly different ($P > 0.3$).

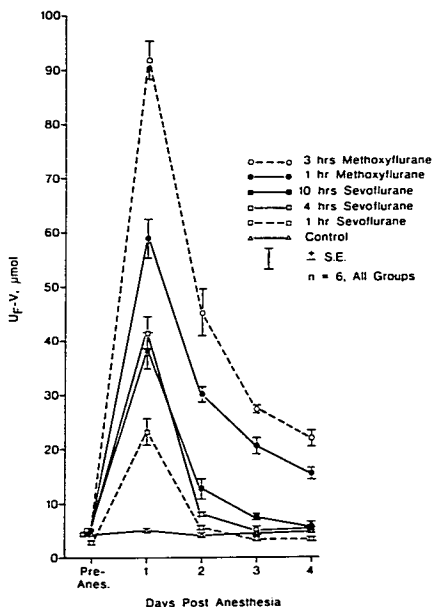
Discussion

Sevoflurane has several properties of possible advantage in clinical anesthesia practice. Its blood-gas partition coefficient is 0.59 and its oil-gas partition coefficient is 42,

so anesthetic induction and emergence should be rapid. This was verified by the present study. All rats were anesthetized within 2 to 3 minutes after introduction of 1.4 per cent sevoflurane into the chamber. Those anesthetized for 1 or 4 hours awakened and righted themselves within 1 or 2 minutes after anesthesia was discontinued; rats anesthetized for 10 hours righted themselves within 15 minutes after the end of anesthesia. Anesthetic induction with 0.5 per cent methoxyflurane required at least 30 minutes, and emergence after 3 hours of anesthesia required approximately an hour. Also, it is of practical importance that the vapor pressure and potency of sevoflurane are such that the Copper Kettle type of vaporizer, now employed for other inhalation agents, can be used for its administration.

Biochemical stability, that is, resistance to

FIG. 2. Twenty-four-hour urinary inorganic fluoride excretion after anesthesia. Cumulative four-day excretion after methoxyflurane was three to four times greater than after sevoflurane. Elevated levels persisted longer after methoxyflurane anesthesia.



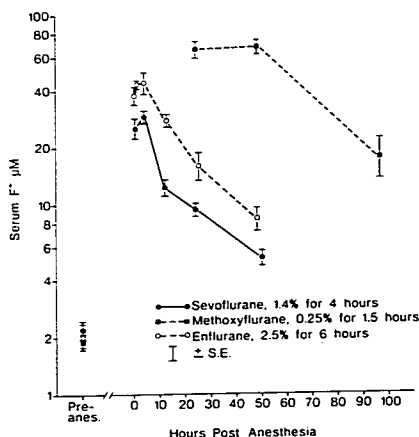


FIG. 3. Serum inorganic fluoride concentration following 4 hours of 1.4 per cent sevoflurane anesthesia. Inorganic fluoride concentration peaked 4 hours after anesthesia and declined rapidly. Decay curves for serum inorganic fluoride following methoxyflurane and enflurane anesthesia are taken from a previous study.⁶

metabolic degradation, is a desirable property of anesthetic agents. This study employs production of inorganic fluoride as an index of biochemical stability. While this metabolic product has been shown to have clinical significance because of dose-related nephrotoxicity following methoxyflurane anesthesia, it may not be the only product of the biodegradation of inhalation anesthetics with significant biologic effect. Furthermore, until the stoichiometry of the degradation of fluorinated anesthetics is better described, the relationship between defluorination and extent of total metabolism will remain poorly defined.

The results of our *in-vitro* study indicate that sevoflurane was defluorinated at a rate similar to methoxyflurane, the least stable of the fluorinated methylethyl anesthetic ethers. If biochemical stability were the only factor in defluorination of inhalation anesthetics, this would be an unacceptable defect. After methoxyflurane anesthesia, however, serum inorganic fluoride levels were more than six times higher and urinary inorganic fluoride excretion was three to four times greater than after comparable exposures to sevoflurane. The most likely explanation for the discrepancy between *in-vitro* and *in-vitro* sevo-

flurane and methoxyflurane metabolic data is that the low tissue solubility of sevoflurane facilitated its rapid postanesthetic pulmonary excretion, whereas the greater solubility of methoxyflurane retarded its elimination. Therefore, sevoflurane was relatively unavailable during the postanesthetic period, the interval during which the majority of anesthetic metabolism occurs. In experiments *in vitro*, where reaction time was fixed at 30 minutes and the drugs were present in equimolar amounts, tissue solubility was not a significant factor in determining defluorination. Data from previous studies with enflurane^{3,5} support the conclusion that low tissue solubility may be the explanation for the differences in sevoflurane and methoxyflurane defluorination. *In vitro*, enflurane is defluorinated at about one seventh the rate of methoxyflurane,⁵ and therefore, it also should be defluorinated at approximately one seventh the rate of sevoflurane. Enflurane has a blood-gas partition coefficient of 1.9 and an oil-gas partition coefficient of 98; it is considerably more soluble than sevoflurane. However, inorganic fluoride excretion following enflurane anesthesia is approximately the same as that following sevoflurane.⁶ Although enflurane,

biochemically, is more stable than sevoflurane, its greater tissue solubility results in slower elimination and, consequently, a similar extent of defluorination.

Nephrotoxicity is related to the duration for which high levels of inorganic fluoride are maintained, as well as the absolute level. Comparison of data from this and a previous *in-vivo* study with enflurane (fig. 3) reveals similar peak values and shapes of the inorganic fluoride decay curves.⁶ Peak inorganic fluoride levels after a lesser methoxyflurane exposure were higher and remained elevated longer than after anesthesia with either of the less soluble drugs. Thus, the nephrotoxic potential of sevoflurane, due to its metabolism to inorganic fluoride, should resemble that of enflurane rather than that of methoxyflurane. In fact, in the present study, there were no real abnormalities after as long as ten hours of sevoflurane anesthesia. To the contrary, as little as one to three hours of methoxyflurane has resulted in mild, polyuric, vasopressin-resistant nephrotoxicity,² whereas six to ten hours of enflurane are required to produce a similar lesion.⁶

The only apparent adverse effect following sevoflurane administration was weight loss, almost 10 per cent of total body weight in rats anesthetized for 10 hours. Almost all of the loss occurred during the first postanesthetic day, when rats did not appear to feed well. However, they did not regain the lost weight during the next three days, when food and water intake and activity returned towards normal. Rats anesthetized with methoxy-

flurane for three hours lost an average of 26 grams the first postanesthetic day, regaining 9 grams by the fourth postanesthetic day. This is the usual pattern in this type of experiment.^{2,6} Liver and kidney sections appeared normal, and no other gross abnormality was apparent at the time of sacrifice. The explanation for the weight loss is not clear.

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Neonatology

ERYTHROBLASTOSIS AND PROMETHAZINE Promethazine hydrochloride has been studied in animals in the laboratory and found to be immunosuppressive. Its use during pregnancies affected by erythroblastosis is reported here. It has not been possible, with this small series of patients, to prove unequivocally its effectiveness in ameliorating the effects of this disease. How-

ever, it is believed that in some individual cases beneficial results have occurred at the dosage used. The probable mechanism of action of this drug *in vivo* is metabolic suppression of the fetal reticuloendothelial system cells. (Cusdon, J. P.: Possible Ameliorating Effects of Erythroblastosis by Promethazine Hydrochloride, *Am J Obstet Gynecol* 117:1101-1108, 1973.)