THE UPTAKE AND DISPOSITION OF ENFLURANE
DURING AND FOLLOWING ANESTHESIA IN OBESE AND NON-OBESE HUMANS

by
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STATEMENT BY AUTHOR

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To my wife Mary Jo, whose encouragement and understanding made this endeavor a success.
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ABSTRACT

The disposition of enflurane, a volatile halogenated anesthetic, was studied in obese (n=24) and non-obese (n=8) informed consenting adult human subjects undergoing elective intraabdominal surgical procedures. Enflurane and fluoride ion, a nephrotoxic metabolite of enflurane, were measured in the blood of subjects prior to, during, and up to forty-eight hours following exposure to 1.9 ± 0.2 MAC hours of enflurane. Arterial enflurane in obese subjects reached maximal concentrations three times faster than non-obese subjects who reached similar blood levels eighty minutes after the initiation of anesthesia. The blood gas partition coefficient for enflurane in the obese was found to be thirty percent lower than that in the non-obese (1.42 ± 0.02 vs 0.99 ± 0.02, p <0.001) and may possibly explain the observed differences in enflurane uptake. The initial rate of serum inorganic fluoride appearance in the obese (5.6 μM/hour) was twice that seen in the non-obese group. The maximum serum fluoride concentration in both groups occurred at two hours post enflurane anesthesia with the obese having a sixty percent higher concentration (28 ± 2 vs 17 ± 3 μM), indicating increased biotransformation in the obese. The maximum rate of urinary fluoride excretion occurred eight hours after the termination of anesthesia. The rate in obese subjects was not statistically different from that in non-obese subjects. Hepatic biopsies from obese subjects demonstrated high...
levels of fatty infiltration which were not related to the increased enflurane biotransformation observed.
INTRODUCTION

General History

Since the introduction of chloroform in 1849 as an anesthetic agent, the search for a safe volatile halogenated anesthetic has continued. Robbins in 1946 demonstrated that fluorinated hydrocarbons were substantially less flammable than existing anesthetics. However, total fluorination completely ablated anesthetic activity.

The first safe and clinically relevant fluorinated anesthetic was fluroxene (2,2,2-trifluoro ethyl vinyl ether) which was developed in 1951 by Ohio Medical Products, division of Airco Inc. Fluroxene was only slightly less explosive than diethyl ether and produced rapid induction, fair muscle relaxation, and satisfactory analgesia.

The introduction of halothane in 1956 rapidly ended the clinical use of fluroxene. Halothane (2-bromo-2-chloro-1,1,1-trifluoroethane) was first synthesized in 1951 and was demonstrated to be both nonflammable and non-explosive. Following the investigation of its pharmacology by Raventos (1956), halothane became a widely used clinical anesthetic agent. Although halothane caused rapid induction and minimal postoperative nausea and vomiting, it has been associated with occasional liver necrosis (Miller, Dwyer, and Klatskin, 1978) and sensitization of the myocardial conductive tissue to catecholamines (Flack and Alper, 1962).
The synthesis in 1958 and subsequent clinical introduction of methoxyflurane (2,2-dichloro-1,1-difluoro ethyl methyl ether) in 1960 provided an anesthetic which was more potent than halothane. Methoxyflurane is nonflammable, non-explosive, and produces excellent muscle relaxation. However, its high solubility results in slow induction and prolonged anesthetic recovery times. In addition, nephrotoxicity characterized by a loss of urine concentrating ability results from a metabolic product of methoxyflurane, fluoride ion (Mazze, Cousins and Kosek, 1972; Cousins and Mazze, 1973; Cousins et al., 1974).

Enflurane (2-chloro-1,1,2-trifluoroethyl-difluoromethyl ether), a structural analog of methoxyflurane, was first synthesized by Terrel in 1963. Following its evaluation by Dobkin et al. (1969), enflurane was marketed in 1972. Enflurane administration results in rapid induction, fair muscle relaxation, and moderate analgesia (Dobkin, 1970). In addition, biotransformation of enflurane was significantly less than that of methoxyflurane (Cousins et al., 1976; Sakai and Takaori, 1978) greatly reducing the hazard of inorganic fluoride induced nephrotoxicity.

**Enflurane -- Physical Chemical Properties**

The physical chemical properties of enflurane are pertinent since it is these properties which determine the uptake, distribution, and ultimate disposition of the anesthetic.

The minimal alveolar concentration (MAC) for enflurane has been determined to be 1.68 percent (Gion and Saidman, 1971). MAC of an
anesthetic defines the lowest alveolar concentration of the anesthetic at which there is no response to painful stimuli in fifty percent of a specific population. Although MAC is similar to an ED_{50}, it must be noted that MAC in no way describes the actual dose of anesthetic received by a subject.

The physical chemical property which is probably the most useful for estimating the rate of uptake, distribution, and elimination of enflurane is the solubility or partition coefficient of enflurane in various biological media. The most commonly used media and partition coefficients determined include blood/gas, oil/water (representing lipid/water), and tissue/blood. The blood/gas partition coefficient of enflurane previously determined to be 1.9 (Dobkin, Heinrich et al., 1968) is one of the most important factors determining the rate of uptake into the blood (Munson and Eger, 1972; Munson and Bowers, 1967).

It is important to note at this point that the partition coefficient does not describe the absolute concentrations existing in two phases, it merely relates the ratio of the concentrations. Consequently, it may serve as an index for estimating the rate at which maximal blood anesthetic concentrations may be achieved (Eger, 1975). Since the rate of anesthetic uptake into the blood is a function of the availability of alveolar anesthetic (Eger, 1975), it may be concluded that a lower blood/gas partition coefficient will result in a more rapid rate of anesthetic uptake by the blood (Eger, 1975). This is because less anesthetic is removed from the alveoli allowing the alveolar anesthetic concentration to reach equilibrium with the inspired anesthetic concentration faster (Eger, 1975).
A value of 1.9 for the blood/gas partition coefficient of enflurane is slightly less than that of halothane, and one seventh that of methoxyflurane (Vitcha, 1971). This explains in part the more rapid induction and emergence from anesthesia previously mentioned for enflurane (Vitcha, 1971).

The oil/water partition coefficient for enflurane is 120 (Vitcha, 1971). This value is significantly less than values of 236 and 825 obtained for halothane and methoxyflurane, respectively (Vitcha, 1971). In much the same way that the blood/gas partition coefficient determines the rate of anesthetic uptake into the blood, the oil/water partition coefficient determines the rate at which enflurane is absorbed into the body lipids (Eger, 1975). Since the oil/water partition coefficient for enflurane is lower than the values for halothane and methoxyflurane it may be concluded that the uptake of enflurane into lipid rich areas, such as fat and brain, would occur at a rate greater than that of the previously mentioned anesthetics (Munson and Eger, 1972).

**Enflurane -- Absorption, Distribution and Excretion**

As previously mentioned, the absorption, distribution, and elimination of enflurane depends greatly upon its physical chemical properties.

Since blood enflurane concentrations are a function of the alveolar enflurane concentration, the rate at which alveolar enflurane concentrations reach equilibrium with inspired enflurane concentrations
is an important factor in determining the rate of enflurane uptake into the blood (Eger, 1975). Torri (1974) demonstrated that when enflurane was administered to dogs at an inspired concentration of 0.5 percent, equilibrium was attained between the inspired enflurane concentration and the alveolar enflurane concentration after approximately fifteen minutes (Fig. 1). When enflurane administration was discontinued following thirty and sixty minute exposures, the elimination curves obtained (Fig. 2) demonstrated that the alveolar enflurane concentration decreased by eighty percent within ten minutes following the termination of anesthesia. This study implies that enflurane is rapidly removed from the body upon termination of exposure.

Magalini, Bondoli, and Scrascia (1973) also investigated the uptake of enflurane in dogs. Enflurane was administered at an inspired concentration of 1.5 percent for a period of 60 or 180 minutes. Whole blood samples were collected at various times throughout anesthesia from the aorta representing entry of the anesthetic into the system; from the inferior vena cava, representing systemic return; and from the jugular, representing return from the brain. During a 180 minute anesthetic administration whole blood enflurane concentrations within the aorta did not begin to level off until sixty minutes. The enflurane concentration in the jugular vein differed only slightly from the aorta while concentrations in the vena cava were significantly less.

Following the termination of administration, enflurane concentrations in the aorta and jugular vein decreased by fifty percent within twenty minutes with the jugular enflurane concentration
Figure 1. Enflurane pulmonary equilibrium curve determined during exposure to 0.5 percent enflurane. -- Ordinate values represent the ratio between the alveolar anesthetic concentration ($F_A$) and the inspired anesthetic concentration ($F_I$) (Torri, 1974).
Figure 2. Enflurane pulmonary washout curves obtained following thirty
and sixty minutes of enflurane anesthesia. -- Ordinate
values represent the ratio between the alveolar anesthetic
concentration ($F_A$) and the alveolar anesthetic concentration
at the termination of anesthesia ($F_{A0}$) (Torri 1974).
slightly greater than the aortic concentration (Fig. 3). Both the jugular and aortic enflurane concentrations approached zero at sixty minutes. The enflurane concentration in the vena cava interestingly enough changed only slightly until sixty minutes following the termination of anesthesia, at which time the concentration rapidly decreased approaching the aortic and jugular concentrations (Fig. 3). The period following the termination of anesthesia in which vena cava enflurane concentrations remain elevated may play an important role in the biotransformation of enflurane (Magalini et al., 1973).

Enflurane Metabolism -- In Vitro

Enflurane, like methoxyflurane, is bound to and metabolized by hepatic microsomal cytochrome P-450 (Ivanetich, Lucas and Marsh, 1979). The form of cytochrome P-450 induced by phenobarbital demonstrates a type I binding spectra with enflurane and may be predominantly responsible for enflurane metabolism. In addition, enflurane has been shown not to interact with cytochrome P-448 (Ivanetich et al., 1979). This cytochrome P-450 dependent metabolism of enflurane has been shown to produce inorganic fluoride and organic fluorine containing metabolites (Chase et al., 1971). Unlike many other halogenated hydrocarbons (Cohen, 1978), no reactive compounds which are capable of binding to cellular macromolecules have been demonstrated to result from in vitro enflurane metabolism (Gandolfi, 1979).

The in vitro kinetic parameters for enflurane biotransformation have been investigated in both rat and man (Hitt et al., 1977). Hitt et al. (1977) quantified inorganic fluoride production and demonstrated
Figure 3. Whole blood enflurane concentrations in the aorta (---); the jugular vein (-----); and the inferior vena cava (-----) of dogs following 180 minutes of enflurane anesthesia. -- (Magalini et al., 1973).
that Km for enflurane with rat hepatic microsomes was 57.8 ± 0.97 μM, and Vmax was 0.96 ± 0.08 nm inorganic fluoride/30 minutes/mg protein. Also, in hepatic microsomes prepared from human heart donors the Km was found to be 72.4 ± 1.2 μM and Vmax was 0.36 ± 0.05 nm inorganic fluoride/30 minutes/mg protein. Since the Km for enflurane metabolism with both rat and human hepatic microsomes is less than ten percent of blood enflurane concentrations during anesthesia (Devaux et al., 1976), it may be inferred that in both rat and man, the hepatic enzymes responsible for enflurane biotransformation are saturated with substrate during anesthesia. However, non hepatic sites of enflurane biotransformation may possibly exist. Enflurane metabolism in organs possessing known biotransformation capabilities, such as lung and kidney (Blitt, Gandolfi, Soltis and Brown, 1979) and skin (Bickers, Kappas and Alvares, 1974), has not been investigated and could significantly influence both the qualitative and quantitative aspects of in vivo enflurane formation.

The organic products of in vitro and in vivo enflurane biotransformation currently remain unknown. Two pathways for enflurane metabolism have been proposed (Cousins and Mazze, 1974; Cohen and VanDyke, 1977). It is thought that the major route of enflurane biotransformation is initiated by oxidative dechlorination which results in the production of inorganic chloride, fluoride, and the formation of 2-difluoromethoxy-2,2-difluoroacetate. A minor pathway for enflurane metabolism may also exist in which the enflurane ether linkage is cleaved to release inorganic fluoride and produce
chlorofluoroacetate. Hitt et al. (1977) using $^{14}$C-difluoromethyl enflurane demonstrated that only one equivalent of inorganic fluoride ion was released per equivalent of enflurane metabolized. Hitt reasoned that since the radiolabel was present on the methyl carbon, if the enzymatic attack occurred at the ether linkage all radioactivity would have been lost due to the formation of volatile compounds. In addition, ether cleavage would have resulted in the release of more than one equivalent of inorganic fluoride per equivalent of enflurane metabolized. It was concluded that the ratio of metabolites observed could only have resulted from attack on the β carbon of the enflurane molecule.

Holaday, Oda and Smith (1978) employing enflurane also labeled with $^{14}$C in the difluoromethoxy position determined that enflurane undergoes in vivo enzymatic degradation at both the β carbon and the ether linkage. The reaction at the ether linkage was found to proceed at a rate equal to one twelfth of the reaction rate at the β carbon. Thus, the pathways for enflurane biotransformation have not been conclusively established, but it would appear that the β carbon of the enflurane molecule is the most likely site for enzymatic attack.

**Inorganic Fluoride Nephrotoxicity**

Several authors (Chase et al., 1971; Barr et al., 1974; Cousins et al., 1976; Maduska, 1974) have demonstrated the appearance of inorganic fluoride in both serum and urine during and following enflurane anesthesia. Inorganic fluoride resulting from enflurane metabolism is of concern because of the nephrotoxicity associated with
elevated serum inorganic fluoride levels produced by the biotransformation of methoxyflurane. In fact, clinical reports of nephrotoxicity following enflurane anesthesia have begun to accumulate (Eichorn et al., 1976). Initial studies upon the effects of inorganic fluoride resulting from methoxyflurane administration indicated that the threshold for the production of fluoride induced renal dysfunction in man was a serum inorganic fluoride level of 50 μM (Cousins and Mazze, 1973). However, more recent studies on the effects of fluoride resulting from enflurane administration have lowered this value to a level of 33 μM (Mazze, Caverly, and Smith, 1977).

The response of the kidney to elevated inorganic fluoride levels is a general diuresis and a loss of urine concentrating ability (Whitford and Taves, 1973; Mazze et al., 1977; and Whitford and Stringer, 1978). Serum inorganic fluoride levels in man ranging from 200 to 300 μM have resulted in a three to four fold increase in urine output (Taves et al., 1970; Mazze, Trudell, and Cousins, 1971). The rat, on the other hand, has shown a 64 percent increase in urine output as a result of serum inorganic fluoride concentrations of 32 μM (Whitford and Taves, 1973). In both rat and man it has been determined that there is no increase in solute excretion in the presence of diuresis induced by fluoride ion (Whitford and Taves, 1973). Hence, urinary osmolality is significantly decreased.

The mechanisms by which elevated serum inorganic fluoride levels produce a decrease in urine concentrating ability are at this point unknown. The most likely mechanisms are a decreased inner
medilary sodium concentration resulting from inhibition of the sodium transport system in the ascending limb of the loop of Henle, an increased permeability of the ascending limb of the loop of Henle to water possibly as a result of fluoride interference with antidiuretic hormone, or an increase in medilary blood flow (Whitford and Taves, 1973).

Since inorganic fluoride ion has been utilized as an indicator of anesthetic metabolism (Hitt et al., 1977; Sakai and Takaori, 1978; Lowry et al., 1977), and has been shown to be associated with nephrotoxicity when serum concentrations exceed 33 μM, an understanding of the pharmacokinetics of inorganic fluoride is essential. Carter, Heerdt, and Acchiardo (1977) demonstrated no difference in maximum serum inorganic fluoride concentrations or time to reach maximum concentrations when eighteen anephric and sixteen healthy patients were anesthetized with enflurane. Since it has been shown that an excess of 50 percent of an infused dose of inorganic fluoride may be sequestered in the skeletal system of man (Largent, 1960; Ekstrand, Ehrnebo, and Boreus, 1978), these results indicate that the major mechanism for removing inorganic fluoride from serum is uptake by bone. The biological half life for inorganic fluoride sequestered in the skeletal system has been estimated, by indirect methods, to be as long as nine years (Largent, 1960).

Enflurane Metabolism -- In Vivo

Enflurane, as previously stated, is biotransformed to inorganic fluoride ion. If inorganic fluoride induced nephrotoxicity were to
occur in the postoperative patient, serious alterations in the pharma-
cookinetics of medications which depend upon renal function for
clearance could result (i.e. aminoglycoside antibiotics). Therefore,
serum inorganic fluoride concentrations resulting from enflurane admin-
istration have been the subject of a substantial amount of research.

Maduska (1974) examined serum inorganic fluoride levels
following enflurane anesthesia in 102 patients. Results indicated
that, unlike methoxyflurane, serum inorganic fluoride levels following
enflurane anesthesia rarely rose above 50 μM and if the level of 50 μM
was attained it was only for very short periods of time. In addition,
no significant increase in serum inorganic fluoride was evident in
patients who had previously been receiving barbiturate medications
which are known to induce hepatic biotransformation systems (Conney
and Burns, 1962; Conney and Burns, 1972).

Hitt et al. (1977) examined four patients who received a mean
enflurane exposure of 4.2 MAC hours. It was found that serum inorganic
fluoride rose to a maximum concentration of 13 μM during anesthesia
and increased to a maximum concentration of 22.5 μM three hours after
the termination of anesthesia. Serum inorganic fluoride levels
remained elevated until approximately ten hours following anesthesia
at which time levels declined and approached preanesthetic values four
days later.

Lowry et al. (1977) demonstrated the existence of a dose
response relationship between the log of the dose of enflurane admin-
istered (MAC hours) and the serum inorganic fluoride levels resulting
from enflurane biotransformation. Three groups of patients received 0.5 MAC of enflurane for 0.5, 2, and 4 hours for a total of 0.25, 1.0, and 2.0 MAC hours respectively. Serum inorganic fluoride concentrations were measured immediately following the termination of anesthetic exposure. Mean serum inorganic fluoride concentrations were 23.6 ± 4.2 µM following 2.0 MAC hours, 17.7 ± 2.8 µM following 1.0 MAC hour, and 10.5 ± 0.9 µM following 0.25 MAC hours of enflurane. In addition to the correlation which was demonstrated between serum inorganic fluoride concentrations and the log dose of enflurane (MAC hours), an excellent correlation existed between the duration of exposure and the serum inorganic fluoride concentrations detected immediately following exposure (r=0.989). Since an alveolar enflurane concentration of 0.5 MAC is associated with an arterial enflurane concentration of approximately 380 µM (Lowry et al., 1977), it would not be unreasonable to expect a linear correlation between duration of exposure and serum inorganic fluoride concentrations. In light of the previously mentioned in vitro kinetic parameters for enflurane metabolism (Km=72.4 µM) (Hitt et al., 1977) it would appear that hepatic enzyme systems responsible for the biotransformation of enflurane are most likely saturated with substrate.

Mazze et al. (1977) exposed 12 male volunteers to 9.6 MAC hours of enflurane anesthesia to assess the effects of prolonged enflurane anesthesia and metabolic products of enflurane on urine concentrating ability. Serum inorganic fluoride was shown to increase at a rate of approximately 2.1 µM/hour until a maximum serum inorganic fluoride
concentration of 33.6 ± 2.8 µM was attained six hours following the termination of anesthesia. In addition, measurements of maximum urine concentrating ability by vasopressin resistance indicated that maximum urine concentrating ability following enflurane anesthesia had decreased to a value 26 percent below preanesthetic values (P<0.01). It was concluded from measurements of renal blood flow and urinary electrolytes that the decrease in urine concentrating ability observed was not related to alterations in glomerular filtration rate or solute excretion.

Factors Influencing Enflurane Metabolism

Since enflurane is biotransformed to inorganic fluoride ion which is associated with diuresis and loss of urine concentrating ability, great emphasis must be placed upon factors which may increase the biotransformation of enflurane. As previously mentioned, prior exposure to compounds which are known to increase hepatic biotransformation capabilities, such as phenobarbital, has not been shown to result in increased in vivo enflurane biotransformation in both rat and man. However, reports of unusually high serum inorganic fluoride levels resulting from enflurane exposure to patients receiving hepatic enzyme inducing compounds are not uncommon (Maduska, 1974; Cousins et al., 1976). Dooley et al. (1979) investigated the effects of hepatic induction in an excess of 100 patients who were repeatedly exposed to ethanol, phenobarbital, phenytoin, or other miscellaneous compounds. In addition, enflurane biotransformation was investigated in patients who were receiving no medication prior to enflurane exposure. It was concluded that prior treatment with known enzyme
inducing compounds did not increase enflurane biotransformation, nor did it increase the risk of developing inorganic fluoride induced nephrotoxicity. However, a more recent study has demonstrated that the antibacterial agent isoniazid is capable of increasing the in vitro biotransformation of enflurane to an extent which may be significant in vivo (250-400 percent) (Rice, Sbordone, and Mazze, 1978).

Another factor which may play a role in increasing anesthetic biotransformation is the presence of excessive obesity. Several authors (Maduska, 1974; Cousins et al., 1976) have detected serum inorganic fluoride levels following enflurane anesthesia in sporadic obese patients which were significantly higher than mean values measured in non-obese patients. Young et al. (1975) investigated the biotransformation of both methoxyflurane and halothane in twenty-one morbidly obese subjects. Serum inorganic fluoride concentrations in obese subjects who received methoxyflurane were found to increase during anesthesia at a rate more rapid (P<0.05) than that reported for non-obese patients. In addition, maximum serum inorganic fluoride concentrations were greater (P<0.05) and occurred sooner following the termination of methoxyflurane anesthesia in the obese patients. Obese patients also demonstrated increases in serum inorganic fluoride during and following halothane anesthesia which has not been reported for non-obese patients. This data suggests an increase in anesthetic biotransformation or variations in inorganic fluoride kinetics in obese subjects.
Possible explanations for increased biotransformation of anesthetics in obese subjects include an increase in hepatic microsomal activity or an increase in the quantity of anesthetic exposed to the enzymatic systems. Young et al. (1975) speculated that hepatic fatty infiltration, which is present in eighty percent of obese subjects (Bray, 1976), may allow for increased uptake of the lipid soluble anesthetic by the liver, thereby exposing more anesthetic to biotransformation systems.

Additional factors which may possibly effect the disposition and pharmacokinetics of xenobiotics in man are age, gender, genetic constitution, and preexisting disease (Vessel, 1977).

**Obesity**

Obesity is defined as an excess of body fat (Bray, 1976). The characterization of a subject as obese or non-obese requires a measurement of body fat and then subsequent application of that measurement to some subjectively predetermined range or standard for acceptable amounts of body fat within a specific population. Body fat may be measured either directly, as with skin fold calipers, or indirectly, utilizing anthropomorphic relationships between body height and weight. Of the indirect methods body mass index (BMI) \[ \text{BMI} = \frac{\text{weight (kg)}}{\text{height}^2 (\text{m})} \] remains the most accurate method for deriving a numerical representation for the quantity of excess body fat upon an individual (Keys et al., 1972).
The effect obesity has on the disposition of xenobiotics in man has received minimal study. Distinct physiologic differences have been reported between obese and non-obese subjects which may significantly influence xenobiotic disposition (Alexander et al., 1963). Obese subjects have an increased cardiac output when contrasted to non-obese subjects (Alexander et al., 1963). This implies an increase in pulmonary perfusion which may allow for a more rapid uptake of anesthetics by the blood (Yamamura, 1968). In addition, splanchnic blood flow is significantly increased in obese subjects (Alexander et al., 1963), possibly supplying more xenobiotics to organs such as the liver which may result in increased biotransformation.

Indirect evidence exists to indicate that obese subjects may have elevated hepatic cytochrome P-450 levels. Rowe and Wills (1976) demonstrated an increased cytochrome P-450 content and rate of oxidative demethylation of aminopyrine in rats fed high fat diets when compared to rats fed low fat diets. Since a correlation exists between obesity and dietary fat content (Fenton and Chase, 1951; Michelson, Takahashi, and Craig, 1955), the possibility that obese subjects possess increased hepatic biotransformation capabilities exists. Additional indirect evidence for hepatic induction in obese subjects is found in studies which clearly demonstrate elevated rates of steroid metabolism in obese subjects (Dunkelman et al., 1964; Garces et al., 1968; Schteingart, Gregerman, and Conn, 1963).

The disposition of xenobiotics in obese subjects may also be influenced by the presence of pathological conditions. Obesity has been
associated with an increased incidence of diabetes mellitus, chole-
lithiasis, cirrhosis, hypercholesterolemia, and hypertension (Vaughan,
1978). In addition, obese subjects may have an associated protein
losing nephropathy (Warnke and Kempson, 1978). However, whether pre-
existing renal disease in obese patients alters the disposition of
xenobiotics, such as halogenated anesthetics, or potentiates the
effects of nephrotoxins, such as inorganic fluoride ion, is not known.
Enflurane, a halogenated, volatile, methyl ethyl ether very
similar in structure to methoxyflurane, has rapidly become a popular
and frequently used anesthetic. Like methoxyflurane, enflurane has
been shown to be metabolized in part to inorganic fluoride ion in both
rat and man. Resulting serum inorganic fluoride levels have been
shown to be associated with a nephrotoxic syndrome which is character­
ized by polyuria. Initially, the threshold for the production of
fluoride induced renal dysfunction in man was thought to be a serum
inorganic fluoride concentration of approximately 50 μM. More
recently this value has been lowered to a level of 33 μM. The metab­
olism of both halothane and methoxyflurane has been demonstrated to be
significantly increased in obese patients when compared to non-obese
counterparts. Presently enflurane anesthesia is utilized extensively
in both non-obese and obese subjects. Moreover, the obese state has
been demonstrated to influence the disposition of other halogenated
anesthetic agents. Thus, these studies were undertaken to clarify the
role of obesity in the disposition of enflurane.
METHODS

**General Protocol**

The general design of this study was a modification of a protocol prepared by Robert W. Vaughan, M.D. and submitted to the University of Arizona Human Subjects Committee for approval. Approval was received from the University of Arizona Human Subjects Committee on 3/31/78.

**Patient Characteristics**

The age, sex, height, and weight of each consenting adult patient was recorded. All patients were in good health and undergoing elective intraabdominal surgical procedures. Preoperative laboratory values were all within normal ranges and were monitored daily throughout the study. In addition, no patient had preexisting renal dysfunction by history or was receiving any medication known to alter renal function.

The body mass index (BMI) of each patient was calculated from the following equation:

\[
BMI = \frac{\text{Weight (kg)}}{\text{Height}^2 \text{ (m)}}
\]

For the purposes of this study subjects possessing a body mass index in excess of 30 were considered to be obese.

Twenty four obese and eight non-obese consenting adult subjects of varying sex were investigated. The number of subjects
within each group was limited by the availability of patients under-
going appropriate surgical procedures within the time interval chosen for this study.

Patient Preparation

Food and water intake were restricted in all patients for at least eight hours prior to surgery and patients were premedicated with diazepam (10-15 mg, orally) and glycopyrolate (0.2-0.3 mg, intramuscularly) 60 to 90 minutes before anesthesia.

Prior to entering the operating room, a 16 gauge intravenous catheter equipped with a three way stopcock was placed in either the dorsum of the hand or anticubital fossa of all patients by a competent anesthesiologist. This cannula allowed for the administration of intravenous fluids and medications. Subjects were then transported to the operating room.

Upon arrival in the operating room, an anesthesiologist placed a 20 gauge catheter attached to a three way stopcock in the radial artery of either arm of each patient following subcutaneous local analgesia. This allowed for continuous monitoring of arterial pressure, measurement of arterial blood gases, and the collection of arterial whole blood samples.

The anesthesiologist accomplished induction with gallamine (120 mg) premedication, preoxygenation (3-5 minutes), sodium thiopental (375-750 mg), succinylcholine (100-160 mg), cricoid pressure, and placement of a cuffed endotracheal tube. Following induction, a foley catheter was aseptically placed in the urinary bladder of all
subjects by members of the operating room nursing staff. This allowed for the collection of urine during and following anesthesia.

Anesthesia was maintained for approximately two hours with enflurane and nitrous oxide:oxygen (50:50). Enflurane administration was discontinued after two hours and subsequent anesthesia was maintained with fentanyl (100-200 µg) and nitrous oxide:oxygen (60:40) until completion of the surgical procedure. Pancuronium bromide (6-10 mg) was employed for skeletal muscle relaxation. Ventilation was mechanically controlled to maintain arterial oxygen tension at 80-130 mm Hg and arterial carbon dioxide tension at 40 ± 5 mm Hg.

A section of poly vinyl chloride tubing (PV 160) with a three way stopcock attached to the distal end was placed into the trachea of all patients during anesthesia. This allowed for the collection of end-tidal gas for enflurane quantification and subsequent MAC hour calculation (Lowry et al., 1977).

**Anesthetic Delivery System**

Enflurane was delivered from an Ohio Medical vaporizer attached to a Harris Lake anesthesia machine. The vaporizer was calibrated with a Beckman LB-2 infra-red gas analyzer.

**Sample Collection**

End-Tidal Gas

End-tidal gas was collected at fifteen minute intervals during anesthesia for enflurane quantification and subsequent MAC hour calculation. Samples were collected from a section of poly vinyl
chloride tubing which had been placed in the trachea of each patient. The three way stopcock at the distal end of the sampling tube was attached to either a Beckman LB-2 infra-red gas analyzer sampling at a rate of 200 ml per minute for direct quantification of anesthetic, or to a 5.0 ml gas tight syringe (Precision Sampling Corp.). The end-tidal sampling tube was cleared with a 1.0 ml plastic syringe prior to filling the gas tight syringe. The end-tidal gas sample collected was subsequently analyzed by a gas chromatographic technique.

Arterial and Venous Whole Blood

Arterial and venous blood were collected during and following enflurane anesthesia in order that whole blood enflurane concentrations could be determined. One ml of arterial and venous blood was collected at ten minute intervals during and for thirty minutes following the termination of enflurane administration. Samples were then collected every thirty minutes until three hours following the initiation of anesthesia, and additional blood samples were collected at 4, 6, 8, 12, 18, and 24 hours following the initiation of anesthesia.

Arterial whole blood samples of 5.0 ml each for serum inorganic fluoride determinations were collected prior to and at thirty minute intervals during and for up to three hours following the initiation of anesthesia. Subsequently, arterial blood was collected at 4, 6, 8, 12, 18, and 24 hours after the initiation of anesthesia. All blood samples were placed in plastic tubes and stored on ice for later analysis.
In addition, sixty ml of heparinized arterial whole blood was collected from non-obese (n=8) and obese (n=4) patients prior to anesthesia in order that the blood/gas partition coefficient for enflurane could be determined.

Urine

Total urine output was collected from all patients and the rate of urinary inorganic fluoride excretion was calculated during the following intervals after the initiation of anesthesia:

- 0 hours - 1 hour
- 1 hour - 2 hours
- 2 hours - 3 hours
- 3 hours - 4 hours
- 4 hours - 6 hours
- 6 hours - 8 hours
- 8 hours - 12 hours
- 12 hours - 18 hours
- 18 hours - 24 hours

Liver Biopsies

Members of the surgical team obtained needle liver biopsies from ten consenting obese patients undergoing enflurane anesthesia in order that the degree of hepatic fatty infiltration could be assessed. Upon receiving the liver specimen, a portion was removed and placed in normal saline for histological preparation by a trained histologist. The remainder of each specimen was sealed in a plastic bag, quick frozen in a dry ice methanol bath, and stored at -80°C for triglyceride analysis at a later time.
**Analytical Methods**

**End-Tidal Enflurane Content and MAC Hour Calculation**

End-tidal enflurane concentrations were determined by the gas chromatographic analysis of end-tidal gas collected during anesthesia. End-tidal gas was injected in 250 µl aliquots via a gas sampling valve attached to a Varian model 1440 gas-liquid chromatograph equipped with thermal conductivity detection (TCD) and a Varian CDS 101 electronic integrator. Separation was attained with a 6 ft. 1/8 in. O.D. stainless steel column packed with 5 percent SE-30 on Varaport 30 (100-120 mesh) (Supelco Inc.). The injection port and detector were maintained at 150°C and 200°C, respectively. The column temperature was maintained at 80°C.

End-tidal enflurane concentrations were determined from an external standard curve which was prepared by the identical analysis of 1.42 and 0.99 percent enflurane primary standard. MAC hours were calculated from the following equation:

\[
\text{MAC hours} = \frac{\text{mean end-tidal enflurane conc.}(\%)}{1.68 \text{ percent enflurane (MAC)}} \times \text{duration of anesthesia (hours)}
\]

**Whole Blood Enflurane Content**

Arterial and venous whole blood enflurane content was determined by the method of Miller and Gandolfi (in press).

Immediately upon collection, 1.0 ml of whole blood obtained from patients undergoing enflurane anesthesia was added to 2.0 ml of
water saturated n-heptane (spectra grade) in two dram vials equipped with Teflon\textsuperscript{(R)} cap liners (Supelco Inc.). The biphasic system was shaken for a period of five minutes and allowed to settle for a minimum of ten minutes. A 100 µl aliquot of the n-heptane phase was injected into the gas chromatographic system previously described for the determination of end-tidal enflurane content. The column temperature was maintained at 80° C for the initial two minutes following injection. Subsequently, the column temperature was programmed to increase at a rate of 50° C/minute until a temperature of 200° C was attained. The column was maintained at 200° C for a period of one minute at which time the column temperature was returned to 80° C.

A calibration curve was prepared by the addition of enflurane to n-heptane previously saturated with water in quantities sufficient to result in five sets of standards, with each set containing standards ranging in concentration from 4.1 to 813 µM enflurane. Enflurane analysis was performed on each set of standards by the previously described method.

Accurate enflurane concentrations in whole blood are difficult to attain due to the extreme volatility of enflurane in aqueous solutions. Therefore, extraction efficiency was estimated following equilibrium between whole blood obtained from human volunteers and n-heptane containing known quantities of enflurane. Duplicate enflurane standards consisting of enflurane concentrations ranging from 8.1 to 8.3 µM were prepared in n-heptane. One ml of whole blood
was then added to one set of standards while the other identical set remained unaltered. Following shaking and equilibration for one hour at room temperature, the n-heptane phases were analyzed for enflurane content. Extraction efficiency was derived from the partition coefficient determined between enflurane content measured in the presence and in the absence of a whole blood aqueous phase.

Analysis of n-heptane containing enflurane in concentrations ranging from 4.1 to 813 μM resulted in a response which was both reproducible (Table 1) and linear (Fig. 4). Linearity was confirmed by linear regression analysis which resulted in a correlation coefficient of 0.9999. Analysis of n-heptane containing equivalent concentrations of enflurane in both the presence and absence of whole blood demonstrated that 98-100 percent of the enflurane in n-heptane remained in the n-heptane phase when mixed with whole blood (Table 2). This was interpreted to mean that an excess of 98 percent of enflurane in whole blood would be extracted into n-heptane.

A typical chromatogram (Fig. 5) resulting from TCD gas chromatographic analysis of an n-heptane extract of whole blood containing enflurane demonstrates the excellent peak resolution obtainable.

Enflurane Blood/Gas Partition Coefficient

Sixty ml of heparinized whole blood was collected prior to anesthesia from four obese and eight non-obese patients. The blood/gas partition coefficient for each collection was determined in the following manner. Five ml of whole blood was placed in each of ten 25 ml reaction flasks (Pierce Chemical Co.). The exact volume of each
Table 1. Peak Areas Resulting from TCD Gas Chromatographic Analysis of Five Sets of Heptane Standards Containing Varying Concentration of Enflurane.

<table>
<thead>
<tr>
<th>Enflurane Concentration(^a) (µM)</th>
<th>Peak Area (µV sec x 10(^{-2}) ±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>6.49±0.22</td>
</tr>
<tr>
<td>8.1</td>
<td>13.50±0.11</td>
</tr>
<tr>
<td>16.3</td>
<td>25.75±0.36</td>
</tr>
<tr>
<td>32.5</td>
<td>50.94±0.73</td>
</tr>
<tr>
<td>65.0</td>
<td>100.83±1.98</td>
</tr>
<tr>
<td>81.3</td>
<td>129.69±1.02</td>
</tr>
<tr>
<td>203.3</td>
<td>332.79±0.34</td>
</tr>
<tr>
<td>406.5</td>
<td>639.85±0.95</td>
</tr>
<tr>
<td>813.0</td>
<td>1288.45±2.61</td>
</tr>
</tbody>
</table>

\(^a\) N=5 for each concentration
Figure 4. Typical calibration curve derived from TCD gas chromatographic analysis of enflurane standards prepared in n-heptane.
Table 2. Determination of the Partition Coefficient of Enflurane between Whole Blood and Heptane as a Measure of Extraction Efficiency. a

<table>
<thead>
<tr>
<th>Initial Enflurane Concentration in Hexane</th>
<th>Peak Area in Heptane</th>
<th>% Enflurane Remaining in Heptane</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blood Plus Heptane b</td>
<td>Heptane only b</td>
</tr>
<tr>
<td>8.1 μM</td>
<td>1804</td>
<td>1628</td>
</tr>
<tr>
<td>162.6 μM</td>
<td>3177</td>
<td>3252</td>
</tr>
<tr>
<td>487.8 μM</td>
<td>9047</td>
<td>9037</td>
</tr>
<tr>
<td>650.4 μM</td>
<td>11739</td>
<td>11860</td>
</tr>
<tr>
<td>813.0 μM</td>
<td>128369</td>
<td>131619</td>
</tr>
</tbody>
</table>

a Refer to methods for analysis technique.

b Peak area units are μV-seconds.
Figure 5. A typical chromatogram resulting from TCD gas chromatographic analysis of whole blood containing enflurane.
flask had previously been determined by water displacement. Each flask was equipped with a screw cap top and a Teflon® coated self sealing septa which allowed for access to the flask contents by syringe. The flasks were sealed and shaken in a controlled environment room maintained at 37°C. After 30 minutes, quantities of enflurane were added to each flask by a micro syringe in amounts sufficient to produce enflurane concentrations in the gas phase which approximated 0.25 to 2.0 MAC (0.4-3.4 v/v percent). Samples were then allowed to equilibrate for an additional thirty minutes, after which time 250 µl of the gas phase was removed from each flask with a 250 µl gas tight syringe (Hamilton Co.). The syringe contents were then subjected to gas chromatographic analysis for enflurane by the method previously described for quantitating enflurane in end-tidal gas. Gaseous enflurane standards were prepared by the addition of enflurane to flasks of known volume which contained no blood. Subsequent analysis of these standards allowed for the preparation of a standard curve.

Enflurane concentrations present in the blood were determined following the removal of 500 µl of whole blood from each flask with a glass syringe equipped with a 21 gauge stainless steel needle. The 500 µl of whole blood from each flask was added to 1.0 ml of water saturated n-heptane in screw cap glass tubes and the mixtures were shaken for ten minutes. The enflurane content in the heptane phase was then determined by the method previously described for the quantification of enflurane in whole blood. The blood/gas partition
coefficient (α) was calculated from the following equation:

\[ \alpha = \frac{\text{blood enflurane concentration}}{\text{gas enflurane concentration}} \]

Urine and Serum Inorganic Fluoride

Urine and the arterial and venous whole blood collected during and following enflurane anesthesia were analyzed for inorganic fluoride content by the following technique. Serum was obtained by centrifugation of coagulated blood at 1000 g for ten minutes in a Sorval table top centrifuge. A wooden probe was then used to remove the fibrous component of the serum. Upon recentrifugation for ten minutes at 1000 g a clear yellowish serum resulted. Inorganic fluoride analysis was then conducted on samples containing 500 µl of serum or urine and 500 µl of ionic strength buffer (TISAB) employing an Orion 96-06 fluoride specific electrode attached to a Corning millivolt meter. In addition, inorganic fluoride analysis was performed upon samples containing 500 µl of TISAB and 500 µl of each of six inorganic fluoride standards (10, 62.5, 125, 250, 500, and 1000 µM) which were prepared from a purchased fluoride standard (Orion Research Inc.). Since a linear relationship exists between the log of the millivolt readings obtained and the inorganic fluoride concentration (Orion Research Inc., 1977), serum and urine inorganic fluoride concentrations were determined by reading the concentration associated with the millivolt readings obtained from the prepared standard curve.
Hepatic Histology and Triglyceride Determination

Liver biopsy specimens obtained from ten obese patients undergoing enflurane anesthesia were investigated as to their degree of fatty infiltration by histological and biochemical techniques. Histologic preparation consisted of dehydration, paraffin infiltration, sectioning, and hematoxylin and eosin staining. The preparations were then viewed and graded as to their degree of fatty infiltration by Samuel Paplanus, M.D. of the Pathology Department, University of Arizona. Grading criteria were as follows:

0 -- no fatty infiltration
1 -- mild fatty infiltration
2 -- moderate fatty infiltration
3 -- severe fatty infiltration

Triglyceride analysis was performed upon liver specimens by a modified Dade method (Goldfinch and Prudhoe, 1975). Liver specimens were weighed and homogenized following the addition of 1.0 ml of 0.005 M phosphate buffer pH 7.0 and 0.5 ml isopropyl alcohol. Triglyceride standard solution (Supelco Inc.) containing 200 mg% triglyceride was diluted with 0.005 M phosphate buffer pH 7.0 to produce five standard solutions containing 0, 50, 100, 150, and 200 mg% triglyceride. Two hundred microliters of liver homogenates and standards were added to tubes containing 1.0 ml of 0.04 M sulfuric acid. An equivalent aliquot of 0.005 M phosphate buffer pH 7.0 was also added to 0.04 M sulfuric acid to form a blank. Triglycerides were extracted with n-heptane:isopropanol (4:7).
Triglyceride extraction efficiency has been determined to be 96-106 percent (Goldfinch and Prudhoe, 1975). Subsequently, 0.5 ml of the triglyceride extract from each tube was transferred to additional tubes which contained 0.5 ml of 0.1 N sodium hydroxide in isopropanol and mixed thoroughly. The mixtures were then left at room temperature for a minimum of five minutes after which 0.5 ml of fresh sodium periodate solution (1.8 x 10^-2 M sodium metaperiodate in 2.0 M acetic acid) was added. The tubes were allowed to sit for two minutes during which time the mixture developed into a biphasic solution. Three ml of freshly prepared color reagent (4.0 ml acetylacetone in 100 ml of 6.0 M ammonium acetate pH 6.0) was then added to all tubes. After mixing, the tubes were incubated at 56° C for ten minutes. Following cooling in cold water, the contents of each tube were read against the reagent blank at 415 nm in a Guilford Stasar II spectrophotometer. A standard curve was prepared using the absorbances read at 415 nm and the triglyceride content in the prepared standards. Milligrams of triglyceride per 100 mg of liver was calculated by the following equation:

\[
\frac{\text{mg triglyceride}}{100 \text{ mg liver}} = \frac{\text{mg triglyceride/100 ml homogenate}}{\text{mg liver/100 ml homogenate x 100 mg liver (wet wt)}}
\]

Enflurane Metabolites — Extraction

Urine ranging in volume from one to three liters was obtained from obese and non-obese patients during and within twenty four hours following enflurane anesthesia. Concentrated hydrochloric acid was added to the urine in amounts sufficient to result in a final
hydrochloric acid concentration of approximately 1.0 M. A volume of
light petroleum ether (boiling point 30-60° C) equal to one fourth the
urine volume was then added to form a biphasic solution. The solution
was shaken for fifteen minutes after which the aqueous layer was
removed. The petroleum ether layer and emulsified material were
separated into two phases by centrifugation for fifteen minutes at
1000 g in a Sorval table top centrifuge. The petroleum ether phase
was placed in a separatory funnel and shaken for twenty minutes with
forty ml of 0.1 N sodium hydroxide. The 0.1 N sodium hydroxide layer
was then removed and the petroleum ether layer discarded. Since the
0.1 N sodium hydroxide layer should contain any acidic metabolites of
enflurane it shall hereafter be referred to as the **acidic metabolite
extract**. The acidic metabolite extract was either lyophilized for gas
chromatographic analysis or subjected directly to liquid anion exchange
chromatography for the resolution of organic acid metabolites of
enflurane.

**Enflurane Metabolites -- Column Chromatography**

Liquid anion exchange chromatography of the acidic metabolite
extract was accomplished with an AG 1-10x 200-400 mesh analytical grade
anion exchange resin. The exchange resin was hydrated and poured into
a glass column (2.5 cm internal diameter) to produce a packed column
bed 56 cm in length. Both ends of the column were sealed with flow
adapters (Pharmacia Fine Chemicals) and connected to a Pharmacia
peristaltic pump. The upper flow adapter was then attached to a Gilson
fraction collector. Conversion of the resin from the chloride salt to the more reactive acetate salt was accomplished by eluting the resin with one liter of 1.0 N sodium hydroxide followed by 300 ml of 1.0 N acetic acid. The column was then rinsed with distilled water until the effluent pH was greater than 4.8. Upon completion of the water wash, the acidic metabolite extract was applied to the column via the peristaltic pump and eluted with one percent potassium bromide (Holaday et al., 1978) at a rate of 0.6 ml per minute. Simultaneously, effluent fractions of eight ml each were collected until a total of forty fractions were attained. Aliquots of 500 µl were removed from each fraction and assayed for inorganic fluoride content by the method previously described for serum and urine. All fractions were then acidified with 1.0 ml of concentrated hydrochloric acid and shaken for ten minutes with 2.0 ml of petroleum ether. The petroleum ether layer was then removed and back extracted with 1.0 ml of 0.1 N sodium hydroxide. The petroleum ether layer was discarded and the 0.1 N sodium hydroxide extracts were lyophilized to dryness. Sodium metal (75-100 mg) was then added to each lyophilizate and the samples were heated with a gas flame until blue sodium gas was observed. At this point organically bound fluoride (i.e. organic enflurane metabolites) in the samples will react with sodium metal to form sodium fluoride (Blitt, Gandolfi and Soltis, 1979). Samples were neutralized by the direct addition of 2.0 ml of 6.0 M sodium acetate pH 6.8 in the presence of continuous air flow to remove any hydrogen gas which may be formed. Inorganic fluoride analysis was then conducted by the
addition of 500 μl of the resulting solutions to 500 μl of TISAB and analyzed with a fluoride specific electrode.

Enflurane Metabolites ---
Gas Chromatography

Gas chromatographic analysis was conducted upon the lyophilized acidic metabolite extract for organic metabolites of enflurane. The acidic metabolite extract was lyophilized to dryness and lyophilizate samples of approximately 1.0 mg were placed in 1.0 ml reaction vials (Supelco Inc.). Approximately 500 μl of 85 percent phosphoric acid was then added to protonate and volatilize acidic enflurane metabolites. The vials were immediately sealed with screw cap tops containing Teflon® lined rubber septums which allowed for gas phase sampling. The vials were then heated at 50° C for twenty minutes. Subsequently, the septums were punctured with a 5.0 ml gas tight syringe equipped with a side port needle. The gas sample was analyzed for short chain (C₂–C₁₀) carboxylic acids by injection on a 6 ft. 1/8 in. O.D. nickel column packed with 10 percent SP-1200 plus one percent phosphoric acid on 80/100 mesh Chromosorb W AW (Supelco Inc.) in a Varian model 3700 gas chromatograph equipped with flame ionization detection. The flow of nitrogen gas through the column was maintained at 30 ml/minute and the injector port, column, and detector temperatures were maintained at 180, 170, and 240° C respectively. Blanks consisting of sodium hydroxide and 85 percent phosphoric acid were analyzed in an identical manner.
Enflurane Metabolites -- Identification

Liquid ion exchange column chromatography was conducted upon the acidic metabolite extract as previously described with the exception that 5.0 ml of each fraction was removed and stored for analysis by mass spectroscopy. Fractions which were determined to contain organically bound fluoride were lyophilized to dryness and subjected to direct probe electron impact mass spectroscopy with a Hewlett Packard model 5930 quadrupole mass spectrophotometer linked to a Hewlett Packard model 2100A data processor.

Column effluent from the gas chromatographic analysis of the lyophilized acidic metabolite extract was subjected to mass spectroscopy in the following manner. The previously described gas chromatographic column was removed and placed in a Hewlett Packard model 5700A gas chromatograph which was linked to the previously described mass spectrophotometer. The injection port, column, and detector port temperatures were adjusted to those previously employed. Helium flow through the column was maintained at 30 ml/minute. Lyophilized acidic metabolite extract was protonated as previously described and a gaseous sample was injected into the gas chromatographic system. Mass spectrophotometer scans of mass to charge ratio 35 to 200 were conducted at seven second intervals.

Since all postulated metabolites of enflurane may produce a difluoromethyl fragment upon electron impact, the mass to charge ratio of 51 was employed as in indicator of enflurane biotransformation.
products. All information obtained was stored by the Hewlett Packard model 2100 A data processor to allow for subsequent background subtraction.
RESULTS

Following the approval of the University of Arizona Human Subjects Committee the uptake and disposition of enflurane, a volatile halogenated anesthetic, was investigated in twenty four obese and eight non-obese patients. All subjects were undergoing enflurane anesthesia in conjunction with elective intraabdominal surgical procedures.

Patient Characteristics and Exposure

Morphological characteristics of both the obese and non-obese groups demonstrated no statistical differences with the exception of age and weight variables (Table 3).

Enflurane exposure to the obese and non-obese groups, as determined by the quantification of end-tidal enflurane, were found to be $1.8 \pm 0.2$ and $1.9 \pm 0.4$ MAC hours respectively.

Arterial and Venous Enflurane Content

Arterial whole blood from the radial artery and peripheral venous whole blood was collected for enflurane analysis during and following enflurane anesthesia in obese and non-obese patients. Analysis of arterial whole blood obtained from obese patients demonstrated that enflurane is rapidly absorbed following exposure. A steady state enflurane concentration of 600-800 $\mu$M was achieved in arterial blood within twenty minutes after the initiation of anesthesia (Fig. 6). Upon termination of enflurane administration,
Table 3. Patient Morphological Characteristics (Mean ± SE).

<table>
<thead>
<tr>
<th></th>
<th>Obese</th>
<th>Non-Obese</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>24</td>
<td>8</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>165.9±0.9</td>
<td>167.0±2.0</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>127.6±6.0*</td>
<td>67.3±1.2</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>45.9±1.7*</td>
<td>23.6±2.0</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>37.7±5.1*</td>
<td>52.3±5.1</td>
</tr>
</tbody>
</table>

* P<0.05 obese vs non-obese
Figure 6. Arterial (○) and venous (●) enflurane content (mean ± SEM) during and following 1.9 ± 0.2 MAC hours of enflurane anesthesia in 24 obese patients.
arterial enflurane concentrations declined in a non exponential fashion.

Thirty minutes following the initiation of anesthesia, venous enflurane concentrations in obese patients attained a steady state concentration of 400–600 μM. Following the termination of anesthesia, venous enflurane levels declined at a rate not statistically different from that in arterial blood. The mean intraoperative arterial enflurane concentration in obese patients was found to be 654 ± 28 as compared to a mean of 437 ± 30 in venous blood (P <0.05).

In non-obese patients, arterial enflurane concentrations increased at a rate much slower than that found in obese subjects (Fig. 7). A steady state arterial enflurane concentration of 525–600 μM was not achieved until eighty minutes following the initiation of anesthesia. On the other hand, venous enflurane levels in the non-obese patients demonstrated the attainment of a steady state concentration (250–600 μM) twenty minutes following the initiation of anesthesia. Following the termination of anesthesia, venous enflurane concentrations declined in a rapid non exponential fashion and were not statistically different from arterial concentrations. The mean intraoperative arterial and venous enflurane concentrations in non-obese patients were not statistically different (412 ± 39 vs 360 ± 24, respectively).

**Enflurane Blood/Gas Partition Coefficient**

The blood/gas partition coefficient for enflurane was determined in arterial blood obtained from obese (n=4) and non-obese (n=8)
Figure 7. Arterial (o) and venous (●) enflurane content (mean ± SEM) during and following 1.9 ± 0.2 MAC hours of enflurane anesthesia in 8 non-obese patients.
patients. Enflurane content was determined in both the blood and gas phases of a sealed system containing enflurane at 37° C and atmospheric pressure. Mean obese blood/gas partition coefficient was determined to be $0.99 \pm 0.02$ (n=28). This value was significantly lower ($P<0.001$) than the non-obese mean blood/gas partition coefficient which was $1.42 \pm 0.02$ (n=88).

**Serum Inorganic Fluoride**

Although both the obese and non-obese groups received statistically similar enflurane exposures in terms of MAC hours, their mean maximum serum inorganic fluoride concentrations statistically differed ($P <0.01$). The mean maximum serum inorganic fluoride concentration in the obese group was found to be $28.0 \pm 2 \mu M$ as compared to $17.0 \pm 3 \mu M$ in the non-obese, both of which occurred four hours after the initiation of anesthesia (Fig. 8).

Although serum inorganic fluoride concentrations in both groups were shown to increase in a linear fashion until the maximum concentration was reached (Fig. 8), the rate of fluoride appearance differed by a factor of two ($2.5 \mu M/hour$, non-obese vs $5.6 \mu M/hour$, obese). The rate at which inorganic fluoride appeared in the serum of obese patients ($5.6 \mu M/hour$) following enflurane anesthesia was similar to that previously reported (Creasser and Stoelting, 1973) for non-obese patients who received methoxyflurane (Fig. 9). Following the attainment of maximum serum inorganic fluoride levels in both the obese and non-obese groups, serum fluoride levels remained maximally elevated and statistically different ($P <0.05$) for four hours. Serum
Figure 8. Serum inorganic fluoride concentrations (mean ± SEM) during and following 1.9 ± 0.2 MAC hours of enflurane anesthesia in twenty four obese (-----) and eight non-obese (------) patients.
Figure 9. Linear regression analysis of mean serum inorganic fluoride concentrations determined during, and until maximum concentrations were reached following 1.9 ± 0.2 MAC hours of enflurane anesthesia in obese (n=24) (Δ) and non-obese (n=8) (○) patients. -- The rate of fluoride production (m) in obese subjects was found to be similar to the rate of inorganic fluoride appearance in non-obese subjects receiving methoxyflurane (Creasser and Stoelting, 1973). Broken lines represent extrapolations.
fluoride concentrations then declined slowly and approached pre-anesthetic values at 48 hours.

Extensive intragroup variability in serum inorganic fluoride levels was observed in the obese group (Fig. 10). The maximum serum inorganic fluoride concentration in the obese group ranged from 16.5 to 54.2 μM. Less than 10 percent of the obese patients studied were found to have maximum serum inorganic fluoride concentrations which were less than 20 μM while 42 percent demonstrated levels which exceeded 30 μM. The accuracy of the mean maximum serum inorganic fluoride concentration determined in obese patients was illustrated by the fact that precisely 50 percent of the obese patients studied demonstrated maximum serum inorganic fluoride concentrations which were in excess of the calculated mean value.

A linear correlation (r=0.44, P <0.05) was found to exist between obese patient age and maximum serum inorganic fluoride concentrations (Fig. 11). Maximum serum inorganic fluoride concentrations were found to increase with age at a rate of 0.38 ± 0.16 μM/year between the ages of 20 and 60.

**Serum Electrolytes and Creatinine**

Although 42 percent of the obese patients studied had maximum serum inorganic fluoride concentrations which were approximately equal to, or greater than levels which have been shown to be associated with a decrease in maximum urine concentrating ability, no statistical differences between preoperative and postoperative serum electrolytes and creatinine values were detected in obese patients (Table 4).
Figure 10. Serum inorganic fluoride concentrations (µM) in twenty four obese patients during and following 1.9 ± 0.2 MAC hours of enflurane anesthesia.
Figure 11. Linear regression plot (r=0.44, P <0.05) between patient age and maximum serum inorganic fluoride concentration (μM F⁻) resulting from 1.9 ± 0.2 MAC hours of enflurane anesthesia in twenty two obese patients. -- Mean maximum serum inorganic fluoride concentrations were found to increase at a rate of 0.38 ± 0.16 μM/year between the ages of 20 and 60.
Table 4. Pre- and Postoperative Serum Electrolyte and Creatinine Values (Mean ± S.E.M.) in Obese and Non-Obese Subjects.

<table>
<thead>
<tr>
<th></th>
<th>Na⁺ (mM/L)</th>
<th>K⁺ (mM/L)</th>
<th>Cl⁻ (mM/L)</th>
<th>HCO₃⁻ (mM/L)</th>
<th>Creatinine mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OBESE (n = 24)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preoperative</td>
<td>141.0±0.6</td>
<td>4.0±0.1</td>
<td>104.0±0.7</td>
<td>27.6±0.5</td>
<td>0.86±0.04</td>
</tr>
<tr>
<td>Postoperative</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>138.1±0.6</td>
<td>4.1±0.1</td>
<td>104.5±0.8</td>
<td>23.6±1.3</td>
<td>0.81±0.04</td>
</tr>
<tr>
<td><strong>NONOBESE (n = 8)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preoperative</td>
<td>140.1±1.6</td>
<td>4.1±0.2</td>
<td>105.6±0.2</td>
<td>25.6±1.1</td>
<td>0.95±0.08</td>
</tr>
<tr>
<td>Postoperative</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>139.0±1.0</td>
<td>4.5±0.3</td>
<td>105.5±1.3</td>
<td>24.3±1.7</td>
<td>0.87±0.14</td>
</tr>
</tbody>
</table>
However, serum electrolytes and creatinine measurements lack the sensitivity required for detecting mild renal dysfunction (Davidsohn and Henry, 1974).

**Urinary Inorganic Fluoride Excretion**

Urine was collected prior to, during, and following enflurane anesthesia in both obese and non-obese patients. Urinary inorganic fluoride excretion in both groups increased from a value of less than 7 μM/hour within the first thirty minutes of anesthesia to 65 μM/hour 3.5 hours after the initiation of anesthesia (Fig. 12). Inorganic fluoride excretion in the non-obese then remained relatively unchanged until 18-24 hours, at which time the rate of inorganic fluoride excretion was 37 μM/hour. The rate of inorganic fluoride excretion in the obese group, on the other hand, rapidly increased from 65 μM/hour at 3.5 hours following the initiation of anesthesia to 162 μM/hour at 7 hours. The excretion rate in obese patients then fell off rapidly to values similar to those in the non-obese group.

**Atypical Obese Patient**

It seems worthy to note that the rate of urinary inorganic fluoride excretion in one obese patient significantly differed from all other patients studied (Fig. 13). The rate of urinary inorganic fluoride excretion was found to increase during and following anesthesia in a manner not inconsistent with that in non-obese patients. The maximum rate of urinary inorganic fluoride excretion was 46 μM/hour eight hours following the initiation of anesthesia.
Figure 12. Rate of urinary inorganic fluoride excretion ($\mu$M $F^-$/hour) (mean ± SEM) during and following $1.9 \pm 0.2$ MAC hours of enflurane anesthesia in obese (n=24) (------) and non-obese (n=8)(-----) patients.
Figure 13. Serum inorganic fluoride concentrations ($\mu$M F$^-$)(-----), urinary fluoride excretion ($\mu$M F$^-$/hour)(-----), and the rate of urine output (ml/hour)(•••••) following 1.42 MAC hours of enflurane anesthesia in one atypical obese patient.
At this point the inorganic fluoride excretion rate dropped to less than 7 \( \mu M \)/hour where it remained for ten hours. Subsequently, the excretion rate increased to 25 \( \mu M \)/hour at 24 hours, 21 \( \mu M \)/hour at 48 hours, and 30.6 \( \mu M \)/hour at 72 hours. Serum inorganic fluoride concentrations in this patient increased from a preoperative level of 5 \( \mu M \) to a maximum level of 37 \( \mu M \) eighteen hours after the initiation of anesthesia, where it remained for a period of not less than six hours. The serum inorganic fluoride concentration then fell to 22 \( \mu M \) at 48 hours. This decrease in urinary fluoride concentrations may indicate a loss of urine concentrating ability. However, since no sensitive tests of urine concentrating ability were conducted, and no significant alterations in urine output were noted during the period of decreased urinary fluoride excretion, no definite conclusions may be drawn.

**Hepatic Histology and Triglyceride Content**

Liver specimens were obtained from ten obese patients undergoing enflurane anesthesia. Portions of each liver specimen were histologically prepared and the degree of fatty infiltration of each was estimated by an accredited pathologist. Of the ten specimens examined five were judged to have severe fatty infiltration, three had moderate infiltration, and the remaining two specimens had mild fatty infiltration (Table 5). To confirm the presence of fatty infiltration, the triglyceride content of each liver specimen was determined. Values ranged from 3.8 to 33.6 mg triglyceride/100 mg liver (wet weight) (Table 5). A direct correlation between histologic score for fatty
Table 5. Maximum Serum Inorganic Fluoride Concentrations and Degree of Hepatic Fatty Infiltration As Indexed by Histologic Score and Triglyceride Content in Ten Obese Patients Following Enflurane Anesthesia.

<table>
<thead>
<tr>
<th>Patient</th>
<th>mg triglyceride per 100 mg liver</th>
<th>Histology*</th>
<th>max. fluoride level (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>3.8</td>
<td>1</td>
<td>24.5</td>
</tr>
<tr>
<td>I</td>
<td>7.2</td>
<td>1</td>
<td>26.9</td>
</tr>
<tr>
<td>F</td>
<td>11.0</td>
<td>2</td>
<td>30.9</td>
</tr>
<tr>
<td>A</td>
<td>17.7</td>
<td>2</td>
<td>36.7</td>
</tr>
<tr>
<td>E</td>
<td>18.9</td>
<td>3</td>
<td>28.0</td>
</tr>
<tr>
<td>D</td>
<td>21.7</td>
<td>2</td>
<td>20.9</td>
</tr>
<tr>
<td>G</td>
<td>22.3</td>
<td>3</td>
<td>26.2</td>
</tr>
<tr>
<td>B</td>
<td>23.9</td>
<td>3</td>
<td>22.2</td>
</tr>
<tr>
<td>J</td>
<td>31.6</td>
<td>3</td>
<td>33.5</td>
</tr>
<tr>
<td>H</td>
<td>33.6</td>
<td>3</td>
<td>26.8</td>
</tr>
</tbody>
</table>

* Histologic grading system:

0 -- no fatty infiltration
1 -- mild fatty infiltration
2 -- moderate fatty infiltration
3 -- severe fatty infiltration
infiltration and liver triglyceride content was observed. No correlation existed between maximum serum inorganic fluoride level in each patient following enflurane anesthesia and the degree of fatty infiltration as indexed by either histologic score or liver triglyceride content.

Isolation and Identification of Enflurane Metabolites

Column Chromatography

The acidic metabolite extract of urine obtained from obese and non-obese patients during and following enflurane anesthesia was subjected to ion exchange chromatography as a means of separating enflurane biotransformation products. Inorganic and organic fluoride analysis was conducted on the column effluent. One inorganic fluoride peak and two peaks containing organically bound fluoride were reproducibly (n=3) demonstrated (Fig. 14). The presence of at least two distinct organic enflurane metabolites may be indicated.

Attempts at mass spectroscopic analysis of lyophilized portions of the column effluent which had been determined to contain organically bound fluoride were unsuccessful due to the extremely low concentration of metabolite(s) in the samples.

Gas Chromatography

Gas chromatographic analysis was conducted upon the lyophilized acidic metabolite extract of urine obtained from obese and non-obese patients during and following enflurane anesthesia. Results
Figure 14. Inorganic (-----) and organic (-----) fluoride content of fractions obtained from liquid ion exchange chromatography of the acidic metabolite extract. The acidic metabolite extract was applied to a column (1x22 cm) containing AG 1-10x 200-400 mesh analytical grade ion exchange resin in the acetate form. The column was eluted with 1% potassium bromide and fractions of eight ml were collected.
demonstrated three distinct peaks which were not present in identically processed urine obtained from volunteers who received no enflurane (Fig. 15). Portions of the lyophilized acidic metabolite extract were then analyzed under identical conditions following relocation of the gas chromatographic column to a gas chromatograph employing mass spectroscopic detection. No major peaks were detected (Fig. 16). Specific ion monitoring for mass to charge ratio 51 (HF$_2$C-) was conducted and results indicated one possible metabolite peak which may have been hidden in the large background which resulted from hydrocarbon contamination (Fig. 16). Since all postulated enflurane metabolites contain a difluorinated terminal carbon (Cousins and Mazze, 1974; Cohen and VanDyke, 1977), mass spectroscopic analysis was performed and following background subtraction a spectrum not inconsistent with that which would be produced by 2-difluoromethoxy-2,2-difluoroacetic acid was revealed (Fig. 17). Specific peaks which may result from the fragmentation of 2-difluoromethoxy-2,2-difluoroacetic acid are listed in Table 6. All other sizable peaks present in the spectra in Figure 17 are common products of hydrocarbon contamination.
Figure 15. Chromatogram resulting from the gas chromatographic analysis of the lyophilized acidic metabolite extract of urine obtained from patients during and for twenty four hours following enflurane anesthesia. — Separation was accomplished with a 6 ft. 1/8" O.D. nickel column containing 10% SP-1200 plus 1% phosphoric acid on Chromosorb W AW. The column temperature was maintained at 170°C and flame ionization detection was employed.
Figure 16. Reconstructed total ionization chromatogram resulting from the gas chromatograph-mass spectroscopic analysis of lyophilized acidic metabolite extract. Mass spectroscopic analysis was conducted at seven second intervals (ordinate). Mass to charge ratio 51 (M=51) was monitored as an indicator of enflurane biotransformation products. One peak (A) which possibly contained a difluorinated terminal carbon was indicated.
Figure 17. Mass spectrogram resulting from the mass spectroscopic analysis of the gas chromatographic column effluent associated with point A on Figure 16. Mass to charge ratio peaks A, B, C, and D are consistent with fragments which might be produced by the fragmentation of 2-difluoromethoxy-2,2-difluoroacetic acid (Table 6).
Table 6. Mass to Charge Ratios and Specific Fragments Which Could Result from the Mass Spectroscopic Analysis of 2-Difluoromethoxy-2,2-difluoroacetic acid. Each fragment is lettered for peak identification in Figure 17.

<table>
<thead>
<tr>
<th>Mass to charge ratio</th>
<th>Structure</th>
<th>Mass Peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>51</td>
<td>-C-F</td>
<td>A</td>
</tr>
<tr>
<td>67</td>
<td>-O-C-F</td>
<td>B</td>
</tr>
<tr>
<td>95</td>
<td>-C-C(^=\text{O})</td>
<td>C</td>
</tr>
<tr>
<td>111</td>
<td>-O-C-C(^=\text{O})</td>
<td>D</td>
</tr>
</tbody>
</table>
DISCUSSION

In the present study, the uptake and disposition of enflurane, a volatile halogenated anesthetic, was investigated in obese and non-obese subjects. Variations in anesthetic uptake and serum inorganic fluoride levels resulting from approximately 1.9 ± 0.2 MAC hours were observed.

Arterial whole blood enflurane concentrations in obese patients reached a steady state after twenty minutes of enflurane exposure as compared to eighty minutes to reach a steady state of comparable concentrations in non-obese patients (Fig. 18). In light of previous studies in dogs (Magalini et al., 1973) which reported that enflurane concentrations in aortic blood did not attain peak concentrations until sixty minutes following the initiation of anesthesia, it would appear that a value of eighty minutes in non-obese patients is not unreasonable.

The more rapid uptake of enflurane into arterial blood of obese patients may result from several factors. Obese subjects have an increased cardiac output when compared to non-obese subjects (approximately 200 percent) (Alexander et al., 1963). This may result in an increase in pulmonary perfusion, and since the total vascular volume would encounter the lungs faster, an increase in the rate of anesthetic uptake may result (Eger, 1974). However, the difference between total circulation time in obese and non-obese subjects is not
Figure 18. Arterial enflurane content (mean ± S.E.M.) in obese (●) and non-obese (○) patients during and following 1.9 ± 0.2 MAC hours of enflurane anesthesia.
nearly of a magnitude which would be large enough to account for the sixty minute difference observed in time to reach steady state.

The enflurane blood/gas partition coefficient is another factor which may account in part for the variation in enflurane uptake observed. Enflurane blood/gas partition coefficient values of $1.42 \pm 0.02$ in the non-obese and $0.99 \pm 0.02$ in the obese are not only statistically different from each other ($P < 0.001$), but they are substantially different from the previously reported value of $1.91$ Dobkin, Heinrich et al., 1968).

The previous study which determined the enflurane blood/gas partition coefficient to be $1.91$ was accomplished by the addition of known amounts of enflurane to closed systems containing gas and whole blood phases. The enflurane concentration in the gas phase was determined and the whole blood enflurane concentration was subsequently calculated. This method is subject to many possible errors. A small error in the addition of enflurane to the system, or in the determination of the gas phase enflurane concentration will be significantly magnified in the subsequent calculations. The blood/gas partition coefficient determinations performed in this study involved the determination of enflurane content in both the gas and the whole blood phases. The addition of precise amounts of enflurane was not necessary and only one mathematical step was performed, thereby minimizing the magnification of errors. Therefore, the blood/gas partition coefficients determined in this study are deemed the more reliable values. As a note in added proof, the enflurane blood/gas partition
coefficient determined for non-obese subjects was recently substantiated by a published report (Holaday and Fiserova-Bergerova, 1979).

Factors which may contribute to the variation in enflurane blood/gas partitioning observed between obese and non-obese subjects are not readily apparent. However, the more rapid uptake of enflurane by obese patients may be explained, at least in part, by the lower blood/gas partition coefficient determined. This is due to the fact that a lower blood/gas partition coefficient results in the more rapid establishment of an equilibria between end-tidal and inspired enflurane concentrations (Eger, 1975).

Results obtained from the analysis of enflurane in venous whole blood revealed that the venous enflurane concentration generally parallels the arterial concentration, but at a lower value in both groups. Mean intraoperative arterial and venous enflurane concentrations were found to be significantly different (P <0.05) in the obese group while no statistical difference was found in the non-obese group. The mean intraoperative arterial venous difference in the obese group was found to be approximately 217 μM. This implies that obese patients may remove significantly more enflurane from the circulating vascular fluid than non-obese patients.

Several mechanisms may explain the increase in tissue uptake observed in the obese patients. The large quantity of excess body fat in the obese patient provides an excellent site for storage of anesthetic agents. Although the concentration of anesthetic in the fat of obese patients may not differ from that in non-obese patients, the
large increase in mass of body fat in obese patients would result in a significant increase in the total amount of anesthetic stored within the fat. An additional explanation for the increased tissue uptake of enflurane observed in obese patients is an alteration in the fat/blood partition coefficient. The fat/blood partition coefficient for enflurane may differ between obese and non-obese subjects in a manner which would allow for an increased uptake of anesthetic from the blood into the fat of obese subjects. No direct evidence has been presented to indicate that variations in the fat/blood partition coefficient exist between obese and non-obese subjects. However, this possibility must be acknowledged, since variations in blood/gas partition coefficient were demonstrated between obese and non-obese patients.

The decline in blood anesthetic concentrations following the termination of anesthetic administration differed little between the obese and non-obese patients. In both groups blood anesthetic concentrations declined in a non exponential fashion. This indicated the presence of a multicompartamental system (Torri, 1974). Exponential analysis of these systems indicated the presence of three independent components in the obese, and due to an insufficient number of blood enflurane concentrations at later time points, only two components in the non-obese (Fig. 19). No significant differences were noted between the enflurane half life in similar compartments in obese and non-obese groups. However, the enflurane half life in each compartment was significantly longer than those determined from pulmonary washout curves (Torri, 1974).
Figure 19. Exponential analysis of whole blood enflurane decay curves (mean ± S.E.M.) following 1.9 ± 0.2 MAC hours of enflurane anesthesia in obese (n=24) (----) and non-obese (n=8) (-----) subjects. Enflurane half life (t 1/2) in similar compartments (A vs B and C vs D) in obese and non-obese patients was found to be not statistically different.
The various compartments shown in Figure 19 are most likely the result of tissues possessing differing vascular perfusion rates. The highly perfused tissues, such as brain, liver, and kidney, would have the most rapid rate of decay in anesthetic concentration (Fig. 19; lines A and B). Following the highly perfused tissues would be the moderately perfused, such as skeletal muscle, (Fig. 19; lines C and D) and finally, the poorly perfused tissues, such as fat (Fig. 19; line E) (Eger, 1974).

Of specific importance is the slow rate at which enflurane concentrations decline in the poorly perfused tissues. The half life of enflurane in fat, as determined from line E on Figure 19, is approximately thirty hours. Since the whole blood enflurane concentration in obese patients was 17.3 \( \mu \text{M} \) sixteen hours following the termination of anesthesia, one may predict that whole blood enflurane concentrations would not fall below 1 \( \mu \text{M} \) until five days later. This would most likely be the case if enflurane were not biotransformed.

The biotransformation of enflurane was studied by monitoring inorganic fluoride ion, the nephrotoxic metabolite of enflurane. Since enflurane is metabolized by the form of cytochrome P-450 which is induced by phenobarbital (Ivanetich et al., 1979), the biotransformation rate is most likely dose dependent (White et al., 1979) until the enzymatic process is saturated with substrate. Hitt et al. (1977) estimated that \( K_m \) for enflurane metabolism in man was a whole blood enflurane concentration of approximately 30 \( \mu \text{M} \). Since \( K_m \) is the substrate concentration where biotransformation is proceeding at one half
of the maximal rate, it may be assumed that there will be no further increase in the rate of enflurane biotransformation when the whole blood enflurane concentration exceeds 60 μM, which is approximately ten percent of intraoperative whole blood enflurane concentration (Fig. 18).

The rate of inorganic fluoride excretion via the kidneys was found to be very low during anesthesia. Therefore, alterations in serum inorganic fluoride levels during anesthesia due to urinary fluoride excretion may be considered to be negligible. A relative estimate of the rate of enflurane biotransformation may then be obtained from the rate at which inorganic fluoride appears in the serum. This assumes that no vast differences in fluoride ion kinetics exists between the subjects under investigation.

Serum inorganic fluoride concentrations were shown to increase in a linear manner in both the obese and the non-obese groups during anesthesia, and until maximum concentrations were reached. The rate at which serum inorganic fluoride concentrations increased in the obese group was found to be twice that in the non-obese group (5.6 vs 2.5 μM/hour). This suggests that enflurane biotransformation in the obese group may have proceeded at a maximal rate which was twice that of the non-obese, if fluoride ion kinetics are similar in both groups.

The mechanism by which enflurane metabolism is increased in obese patients is unclear. It has been postulated that fatty infiltration of the liver, which is present in eighty percent of all obese subjects (Bray, 1976), may result in an increased anesthetic uptake by
the liver and subsequent increased exposure of enflurane to metabolizing enzymes in obese patients (Young et al., 1975). This mechanism could result in an increase in anesthetic metabolism only if the enzymes responsible for the biotransformation of enflurane were not already saturated with substrate in the absence of fatty infiltration of the liver. On the other hand, fatty infiltration of the liver could result in an increase in enflurane biotransformation if substrate inhibition occurred. Enflurane exposure to hepatic metabolizing systems could be decreased due to enflurane uptake by intrahepatic fat. This could result in a decrease in substrate inhibition and an increase in the rate of metabolism. The results of comparisons between serum inorganic fluoride levels in obese patients during and following enflurane anesthesia and the degree of hepatic fatty infiltration as indexed by histologic score and triglyceride content demonstrated neither a positive nor a negative correlation. It may then be concluded that hepatic fatty infiltration probably plays no role in the increased biotransformation of enflurane observed in obese patients.

Another factor which may contribute to the increased metabolism of enflurane which was observed in obese patients is the possibility that obese patients have elevated cytochrome P-450 levels. Data obtained from rats which were fed high and low fat diets indicated that a diet which is high in fat may cause the proliferation of hepatic biotransformation systems (Rowe and Wills, 1976). Since obese patients may have diets which are high in fat (Bray et al., 1978) the possibility of elevated cytochrome P-450 levels exists. In addition, elevated rates
of endogenous steroid metabolism have been demonstrated in obese patients (Dunkelman et al., 1964; Garces et al., 1968; Schteingart et al., 1963). Thus, the possibility of elevated cytochrome P-450 levels in obese subjects exists and could explain the increased biotransformation of enflurane observed.

A previous report (Hitt et al., 1977) has estimated that Km for enflurane metabolism in non-obese patients is approximately 30 µM. This was accomplished by determining whole blood enflurane levels when urinary inorganic fluoride excretion was at one half of the maximal rate. Hitt et al. assumed that the rate of urinary inorganic fluoride excretion was a constant function of the rate of inorganic fluoride production. Data from enflurane anesthesia in obese patients demonstrates that one half of the maximum rate of urinary inorganic fluoride excretion occurred eighteen hours following the termination of anesthesia, and that enflurane whole blood concentrations at this time were 17.3 µM (Fig. 20). Hence, in obese patients Km for enflurane biotransformation is approximately 17 µM. This indicates that at whole blood enflurane concentrations as low as 35 µM biotransformation of enflurane may be proceeding at a maximum rate. Since it is not uncommon for Km to decrease with induction (Ivanetich et al., 1979; Grundin, Jakobsson, and Cinti, 1973), the variation between Km determined in obese patients and that previously estimated for non-obese patients (Hitt et al., 1977) may possibly be interpreted as an indication of induction in obese patients.
Figure 20. Estimation of the in vivo $K_m$ for enflurane metabolism in obese subjects. $K_m$ was estimated by determining the mean whole blood enflurane concentration ($\mu$M) when mean urinary inorganic fluoride excretion ($\mu$M F$^-$/hour) was at one half of the maximum rate. Mean serum inorganic fluoride concentrations ($\mu$M F$^-$) are included demonstrating that urinary inorganic fluoride excretion reflects serum inorganic fluoride levels.
As previously mentioned, serum inorganic fluoride concentrations in obese and non-obese patients undergoing enflurane anesthesia increased in a linear manner until maximum concentrations occurred two hours following termination of anesthesia. Magalini (1973) demonstrated that the enflurane concentration in the inferior vena cava changed little until seventy minutes following the termination of three hours of enflurane anesthesia in dogs. This may possibly explain why serum inorganic fluoride concentrations continued to increase until two hours following the termination of anesthesia in both the obese and non-obese groups. In addition, mean arterial and venous enflurane concentrations in both groups in this study were found to be less than 60 μM (twice Km) at time points after the attainment of maximum serum inorganic fluoride concentrations (Fig. 18).

A correlation was demonstrated in the obese group between patient age and maximum serum inorganic fluoride level following enflurane anesthesia (Fig. 11). It was found that maximum serum inorganic fluoride concentrations increased at a rate of 0.5 ± 0.2 μM/year between the ages of 20 and 60. This age effect is substantiated by previous studies which demonstrated that younger rats were more resistant to fluoride toxicity than older rats (Maynard, Downs, and LeSher, 1951; Mornstad, 1975). More extensive uptake of fluoride by calcified tissues in young rats has been speculated to be responsible for the age related variations in fluoride toxicity observed (Mornstad, 1975) and is most likely the mechanism of the age effect in this study.
It would appear that the mean maximum serum inorganic fluoride concentration resulting from enflurane anesthesia in both obese and non-obese patient is determined by two factors. The first factor is the rate at which inorganic fluoride appears in the serum, which is a function of the rate of anesthetic metabolism, age, and skeletal affinity for inorganic fluoride. Second is the duration of hepatic exposure to anesthetic in concentrations exceeding twice $K_m$ (60 $\mu$M). If the rate at which inorganic fluoride appears in the serum is constant throughout prolonged enflurane exposures, the maximum serum inorganic fluoride concentration should be predictable.

The results of previous studies (Mazze et al., 1977; Cousins et al., 1976) have demonstrated that the rate at which mean serum inorganic fluoride concentrations increase to maximal values in non-obese patients is approximately 2.1 $\mu$M/hour. Linearity has been demonstrated up to at least fourteen hours (Mazze et al., 1977). A rate of 2.5 $\mu$M/hour was obtained in this study, thereby firmly establishing the rate at which mean serum inorganic fluoride concentrations in non-obese patients increase to maximum levels at approximately 2.0-2.5 $\mu$M/hour. Thus, the only variable for predicting mean maximum serum inorganic fluoride concentration resulting from enflurane anesthesia in non-obese patients is the duration of exposure. An extensive review of the existing literature has revealed only three additional studies in which serum inorganic fluoride concentrations were determined in human subjects following enflurane exposures of known duration (Lowry et al., 1977; Cousins et al., 1976; Mazze et al.,
1977). When the results of these studies were pooled with the results obtained in this study for non-obese patients, it was determined that a linear correlation existed between the mean maximum serum inorganic fluoride concentration and the duration of anesthetic exposure \((r=0.99)\) (Fig. 21). The equation of the linear regression line represented in Figure 21 which may allow for the estimation of mean maximum serum inorganic fluoride concentrations resulting from enflurane anesthesia in non-obese patients is as follows:

\[
\text{Mean Maximum Serum Fluoride} = 2.96 \, \mu\text{M/hour} \times \text{exposure duration} + 10.6 \tag{1}
\]

If a similar equation were to be prepared for obese patients employing \(5.6 \, \mu\text{M/hour}\) (Fig. 9) as the rate constant for the appearance of serum inorganic fluoride, the resulting equation would be as follows:

\[
\text{Mean Maximum Serum Fluoride} = 5.6 \, \mu\text{M/hour} \times \text{exposure duration} + 10.6 \tag{2}
\]

When equation (2) is applied to the obese patients investigated in this study, the mean maximum serum inorganic fluoride concentration that is calculated is \(22 \, \mu\text{M}\) as compared to an actual maximum concentration of \(28 \, \mu\text{M}\). This indicates that equation (2) is at best a low estimate of mean maximum serum inorganic fluoride concentrations resulting from enflurane anesthesia in obese patients. However, when equation (2) is used to approximate the maximum serum inorganic fluoride concentration that might result from the administration of enflurane to obese patients for a period of four hours, a value of \(33 \, \mu\text{M}\) results.
Figure 21. Linear regression plot ($r=0.99$) of maximum serum inorganic fluoride concentrations (mean ± S.E.M.) in non-obese patients and duration of anesthetic administration compiled from several studies. — Data was obtained from this study (a), Lowry et al. (1977)(x), Cousins et al. (1976)(y), and Mazze et al. (1977)(z). The equation of the linear regression line was found to be $y = 2.96x + 10.6$. Maximum serum inorganic fluoride concentration (mean ± S.E.M.) is also shown for obese patients (b) following approximately two hours of enflurane anesthesia.
Since equation (2) is only a low estimate of maximum serum inorganic fluoride concentrations in obese patients, the actual mean serum inorganic fluoride concentration following four hours of enflurane anesthesia in obese patients may well exceed 33 \( \mu M \). In light of the fact that fluoride induced nephrotoxicity has been demonstrated at serum inorganic fluoride concentrations as low as 33 \( \mu M \) (Mazze et al., 1977), it is concluded that enflurane exposures to obese patients exceeding four hours should be avoided. In addition, large variations in serum inorganic fluoride levels during and following enflurane anesthesia in obese patients was observed in this study (Fig. 10). This indicates that significant deviations from the mean are not uncommon, and maximum serum inorganic fluoride concentrations which are in excess of twice the predicted mean value may occur.

The role that urinary inorganic fluoride excretion plays in the attainment and maintenance of serum inorganic fluoride concentrations following enflurane anesthesia is currently unclear. A previous study (Carter et al., 1977) of serum inorganic fluoride levels resulting from enflurane anesthesia in anephric patients and patients with normal renal function demonstrated that renal fluoride excretion plays no major role in the determination of maximum serum inorganic fluoride concentrations. However, data resulting from this study may indicate the contrary.

The rate of urinary inorganic fluoride excretion in one atypical obese patient was shown to fall to less than 10 \( \mu M/\)hour for a period of six to sixteen hours following the termination of enflurane
anesthesia. Serum inorganic fluoride concentrations, on the other hand, were shown to increase in a linear manner until four hours following anesthesia (Fig. 9). Serum inorganic fluoride levels then remained relatively constant throughout the period of decreased renal excretion. Whether in fact serum inorganic fluoride levels remained elevated due to the decrease in urinary inorganic fluoride excretion is not known. However, the fact that serum inorganic fluoride concentrations remained constant in the presence of decreased urinary fluoride excretion may indicate that urinary fluoride excretion plays a role in determining the duration of elevated serum inorganic fluoride levels. The etiology of the period of decreased urinary fluoride excretion may be questioned. This period may represent fluoride induced nephropathy, but in the absence of a sensitive index of urine concentrating ability, such as vasopressin resistance tests, no conclusions may be drawn.

The results obtained from the liquid ion exchange chromatography of extracts of urine obtained from patients undergoing enflurane anesthesia were very interesting. Two separate organic fluorinated metabolites of enflurane were indicated. This evidence tends to confirm previous work (Hitt et al., 1977; Holaday et al., 1978) which indicated that enflurane may be enzymatically attacked at two sites. These sites are the $\beta$ carbon and the ether linkage (Fig. 22). It has been predicted that attack on the $\beta$ carbon would yield 2-difluoro-methoxy-2,2-difluorooacetate as the sole metabolite containing organically bound fluoride (Hitt et al., 1977). The results of gas chromatographic separation and subsequent mass spectroscopic analysis of lyophilized
Figure 22. Proposed pathways for the biotransformation of enflurane. — (Cousins and Mazze, 1974).
urine extracts from patients who received enflurane anesthesia has tentatively indicated the presence of 2-difluromethoxy-2,2-difluoroacetate. The presence of this metabolite confirms the existence of the pathway for enflurane biotransformation involving attack upon the β carbon. The structure of the second organic metabolite as yet remains unknown.
CONCLUSIONS

The conclusions of this investigation are as follows:

1. Marked obesity (body mass index >30) is associated with an increased rate of enflurane uptake. This is due, at least in part, to alterations in the blood/gas partitioning of enflurane in obese subjects.

2. Marked obesity is associated with an increase in the total quantity of enflurane removed from the vascular fluid and stored in the fat.

3. Although enflurane uptake differs, non-metabolic elimination of enflurane is similar in obese and non-obese subjects.

4. Marked obesity is associated with a significant increase in the rate at which enflurane is biotransformed to nephrotoxic inorganic fluoride ion. In fact, it is estimated that in excess of 50 percent of obese patients who receive an enflurane exposure of approximately four hours in duration with experience subclinical fluoride induced nephrotoxicity.

5. Maximum serum inorganic fluoride levels resulting from enflurane biotransformation are directly correlated with the duration of anesthetic administration and are influenced to a lesser degree by subject age.

6. Contrary to previous speculation (Young et al., 1975), hepatic fatty infiltration plays no role in the elevated rate of enflurane
biotransformation observed in obese subjects. Although the actual mechanism by which enflurane biotransformation is increased in obese subjects is unknown, indirect evidence indicates that enflurane biotransformation in obese subjects may proceed at a more rapid rate due to the presence of elevated hepatic cytochrome P-450 levels (Dunkelman et al., 1964).

7. Enflurane biotransformation in man results in the formation of at least two organic metabolites, one of which is most likely 2-difluoromethoxy-2,2-difluoroacetate. The presence of 2-difluoromethoxy-2,2-difluoroacetate confirms the proposed (Cousins and Mazze, 1974) enflurane biotransformation pathway involving oxidative dehalogenation at the β carbon of the enflurane molecule.
REFERENCES


Gandolfi, A. J. Associate Professor of Toxicology, The University of Arizona. Personal communication, 1979.


