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Uptake, Distribution, and Excretion of Fluorocarbon FX-80 (Perfluorobutyl Perfluorotetrahydrofuran) during Liquid Breathing in the Dog

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The uptake, distribution, and excretion of FX-80, a water-insoluble mixture of isomers of perfluorobutyl perfluorotetrahydrofuran, were studied in 17 dogs subjected to liquid breathing with oxygenated FX-80. The absence of excess fluoride ion in urine suggests that FX-80 is not metabolized, nor is it excreted in urine unchanged. Its distribution in the body depends on the lipid content of tissues. The uptake and desaturation curves are multiple exponential functions, the rate constants of which can be predicted from the ratios of lipid contents of blood and tissues and the fractions of cardiac output supplying the tissue compartments. During liquid breathing the concentration of FX-80 in arterial blood averages 0.43 mg/100 ml. Calculations indicate that at saturation a 13.8-kg dog would absorb 1.25 g of FX-80. Of this amount, 71 per cent would be deposited in adipose tissues and bone marrow, 25 per cent in muscles and skin, and 4 per cent in parenchymatous tissues. Desaturation is delayed because deposits of liquid FX-80 are sequestered in the lungs following liquid breathing. (Key words: Uptake, distribution and excretion; Fluorocarbon; FX-80; Liquid breathing; Mathematical model.)

RECOGNITION of the high solubility of gases in chemically inert perfluorinated organic liquids has led to attempts to utilize these liquids as oxygen-transport media.[1] One of these, FX-80 (a mixture of isomers of perfluorobutyl perfluorotetrahydrofuran), has been used as a substitute for air to ventilate the lungs of dogs for periods as long as eight hours.[2, 3] This report describes the uptake, distribution, and elimination of FX-80 in dogs during and following liquid breathing.

Methods

Seventeen mongrel dogs, average weight 13.8 kg (SD 1.3), were subjected to liquid breathing of FX-80. The methods for ventilation of the lungs with fluorocarbon liquid and monitoring and maintaining homeostasis have been described.[2, 3] Briefly, after preoxygenation, the dogs were ventilated three to five times per minute with tidal volumes of approximately 400 ml of oxygenated fluorocarbon liquid. To terminate liquid breathing, fluorocarbon liquid was drained from the lungs by gravity, and the animals were given first oxygen, then air, to breathe.

The dogs were divided into three groups with respect to duration of exposure to fluorocarbon and to the length of time they were permitted to survive after termination of liquid breathing. Group I was made up of three dogs which were anesthetized with sodium pentobarbital, paralyzed with succinylcholine, iv, and ventilated with fluorocarbon for eight hours. One dog was sacrificed immediately at the end of liquid breathing by iv injection of saturated KCl, and samples of various tissues were obtained shortly after death for analysis of fluorocarbon content. The other two dogs were returned to breathing air for long-term follow-up.

Eight dogs comprised Group II. They were subjected to liquid breathing for an hour, after which the fluorocarbon was drained from the lungs by gravity and the animals were allowed

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to breathe oxygen. Blood fluorocarbon levels were monitored for three hours after cessation of liquid breathing, after which the animals were sacrificed and tissues obtained for study of the distribution of fluorocarbon in the body.

In Group III, six dogs were ventilated with fluorocarbon for an hour, as in Group II, but were allowed to survive for ten days. They were returned to breathing oxygen until they were able to maintain Pa_{O_2} values within a safe range, and then to breathing air for ten days. After ten days the animals were sacrificed and tissues obtained for study of the excretion of fluorocarbon. Half of the animals in Groups II and III were paralyzed with succinylcholine during liquid breathing.

Analytical Procedures

Blood and urine samples were drawn and stored anaerobically. One ml of blood or 10 ml of urine were extracted on the day of sampling with 2 ml of hexane in 10-ml glass-stoppered centrifuge tubes.

Tissue samples were immediately sealed in Mylar film and frozen until analyzed. Approximately 0.5 g of tissue was ground in a Dual glass tissue grinder with 4 ml of hexane and dried by addition of 2 g of anhydrous sodium sulfate. According to the concentration of FX-80 in the sample, 0.2 to 1.0 μl of extract was injected into the chromatographic column. Gas chromatography was carried out on a Microtek model GC-2500R, equipped with a tritium electron-capture detector (applied voltage was 9 volts). The column was 10 per cent silicone DC-200/12,500 on Chromosorb W, AW, DMCS, 60–80 mesh, in glass tubing ¼ inch × 9 feet. The temperatures were routinely: inlet 105 C, column 60 C, detector 180 C. Carrier gas was nitrogen at 18 ml/min, pressure 25 psig. The amount of FX-80 was evaluated from the area of the first three major peaks using a disc chart integrator.

Fluoride concentrations in urine were determined with a Beckman fluoride-ion-activity electrode; readings were made on a Radiometer potentiometer.[4]

Blood and tissue lipids were extracted into a chloroform–methanol mixture (2:1), dried, and measured gravimetrically using a Cahn micro electrobalance.

Results

Every dog survived ventilation with liquid fluorocarbon. Pa_{O_2} remained above 100 torr during liquid breathing; however, hypercarbia occurred. Arterial *p*H was maintained above 7.2 during eight-hour periods of liquid breathing by infusion of THAM-E. Pa_{CO_2} returned to normal after reconversion to breathing air. Detailed results of blood–gas analyses have been reported.[2, 3]

Gas chromatograms of FX-80 extracted into hexane from the tissues of all exposed dogs had profiles different from that of a standard solution of FX-80 liquid (figs. 1, 2). Blood, muscle, and other tissues, with the exception of lung, yielded essentially identical chromatograms, in which the components having relatively long retention times were enhanced. Samples obtained from the lungs ten days after exposure showed enhancement of the components that had shorter retention times and attenuation of those with long retention times (fig. 2).

The concentration of FX-80 in arterial blood increased rapidly at the beginning of liquid breathing, approaching a steady-state plateau in 15 minutes (fig. 3). The average steady-state concentration in the arterial blood of 17 animals was 0.43 mg/100 ml (SD 0.09); however, the concentration in a given animal fluctuated 20 per cent above and below its average during the period of liquid breathing. A line was fitted to the experimental values by the method of least squares, using a Hewlett-Packard desk computer, model 9100A. The equation of the line, which describes the time course of saturation of arterial blood in 17 dogs, is:

$$c_a = 0.43 - 0.34e^{-0.16t} \qquad (1)$$

where c_a is the concentration of FX-80 in arterial blood in mg/100 ml t minutes after onset of liquid breathing (fig. 3).

The concentration of FX-80 in mixed venous blood approached that of arterial blood in an exponential manner. Figure 4 illustrates a representative experiment in liquid breathing in which two decay constants could be distinguished.

In contrast to the rapidity of the saturation process, desaturation proceeded slowly, ac-

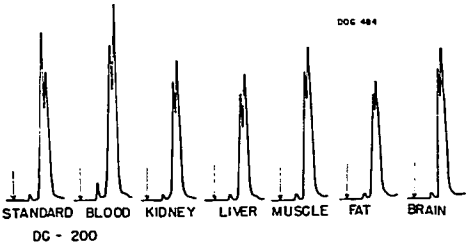

FIG. 1. Gas chromatograms of FX-80 diluted in hexane (standard) and hexane extracts from tissues of a dog sacrificed three hours after a period of liquid breathing lasting an hour. The first major peak of FX-80 is attenuated in the extracts relative to the second major peak. Column packing: 9-ft × ¼-in DC-200/12,500 at 60 C, N_2 at 18 ml/min electron-capture detector.

cording to the equation computed from the data obtained in the six dogs of Group III (fig. 5):

$$c_v = 0.29e^{-0.99t} + 0.14e^{-0.20t} \qquad (2)$$

where c_v is venous concentration in mg/100 ml at time, t, measured in *days* after discontinuance of liquid breathing (fig. 5).

Concentrations of FX-80 found in blood and tissues are given in table 1. Blood concentrations had decreased insignificantly three hours after termination of liquid breathing. The concentrations found in the vessel-rich organs (liver, kidney, ovary, brain gray matter) of Group II dogs, subjected to liquid breathing for an hour and sacrificed three hours later, differed little from those of a Group I animal exposed for eight hours and sacrificed immediately. The concentrations in muscle, adipose tissue, and brain white matter of the Group I dog were considerably higher than those obtained from Group II and Group III dogs. Depletions of FX-80 from vessel-rich organs and muscle of Group III dogs after ten days of survival were 95 and 80 per cent complete, respectively. The concentration of FX-80 in adipose tissue appeared to have increased threefold during the ten days of desaturation when compared with concentrations found in adipose tissues of Group II dogs. The lungs of two dogs exposed to liquid breathing for an hour and sacrificed ten days later contained much higher concentrations of FX-80 than were found in any other tissues sampled during these studies.

The concentrations of FX-80 found in the tissues of a dog exposed for eight hours, with

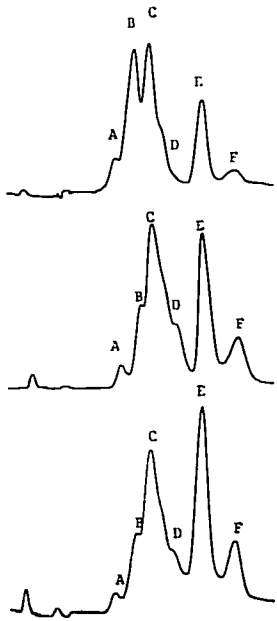

FIG. 2. Gas chromatograms of hexane extracts of FX-80 from lung (top tracing) and muscle (bottom tracing), and a hexane solution of FX-80 (center tracing). Tissues were obtained from a dog sacrificed ten days after a period of liquid breathing lasting an hour. The letters designate peaks having identical retention times. Column conditions are as in figure 1, except that column temperature was 23 C.

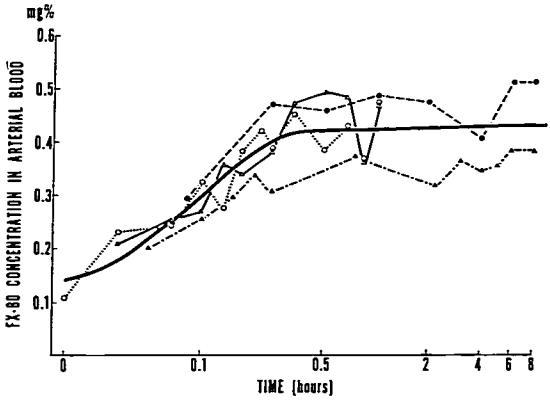

FIG. 3. Saturation of arterial blood with FX-80 during liquid breathing. The heavy line represents the computed average of values for 17 dogs. The interrupted lines connect values obtained from four dogs, two subjected to liquid breathing for an hour (open symbols) and two for eight hours (solid symbols).

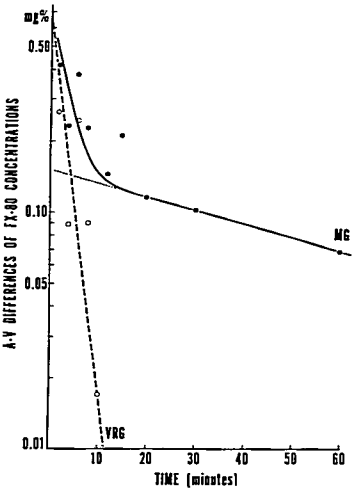

FIG. 4. Arterial–venous differences during uptake of FX-80 in a representative dog. The differences between simultaneous arterial and venous blood concentrations of FX-80 (filled circles, solid line) are plotted against time. The solid line appears to be the sum of two exponential functions (dashed lines).

the exception of adipose deposits, were proportional to the lipid contents of these tissues (fig. 6). Two dogs whose livers contained much higher concentrations of FX-80 than the livers of the other members of their groups were found to have abnormally fatty livers.

FX-80 was present in urine in trace quantities only, *i.e.*, less than 1 μg/100 ml. The concentrations of fluoride ion in urine samples did not exceed normal values, which are less than 1 μg/ml.

Discussion

Two features of the present study distinguish it from similar studies of uptake via the lungs of common organic solvents and volatile anesthetic drugs. These are: 1) the physical and chemical properties of FX-80 liquid, which include insolubility in water, extreme chemical stability, and resistance to biotransformation; 2) the presentation of the material to the body at its full vapor pressure at body temperature (50 torr at 35 C) by filling the lungs with undiluted liquid.

FX-80 is a mixture of isomers of perfluorobutyl perfluorotetrahydrofuran having a boiling point of 102 C. We could not completely separate the components of the mixture with gas chromatography. Seven peaks were suffi-

ciently separated, however, to permit estimation of their retention times and relative quantities (fig. 2). Peak areas obtained with an electron-capture detector are not representative of absolute quantities of components of the mixture, since sensitivity of the electron-capture detector depends on the electron affinity of the compound. Electron affinity varies widely within chemically-related species. When analyzed with a thermal conductivity detector, FX-80 appeared to consist mainly (91 per cent) of three compounds that have relatively short retention times, whereas these three components contributed only 60 per cent of the total peak area of the electron-capture chromatogram.

Components of the mixture having relatively long retention times are concentrated in tissues of the body other than the lungs, and components that have shorter retention times accumulate in the lungs (figs. 1, 2). Also, a linear correlation between retention times on a non-polar column packing and experimentally determined blood–gas partition coefficients of the components was observed (fig. 7). Both rate of uptake and retention time are determined by liquid–gas partition coefficient.[5, 6] It seems, therefore, that more rapid uptake from the lungs of components having high blood–gas partition coefficients explains the differences in the relative peak heights of FX-80 extracted from lungs of exposed dogs, FX-80 from blood and other tissues, and a standard solution of FX-80 in hexane. Furthermore, the ratios of peak heights of extracts of tissues other than lung were the same as those of extracts of blood, indicating that the ratios of the tissue–blood partition coefficients of all components of the mixture were similar in all tissues.

Excretion in exhaled air must be the principal route of elimination of FX-80, in view of its finite vapor pressure, resistance to biodegradation (*vide infra*) and minute concentrations in urine. The observed presence of high concentrations of FX-80 in the lungs ten days after liquid breathing is not predicted by current theories of uptake and excretion of liquid-soluble vapors.[6, 7] One possible explanation is that droplets of liquid FX-80 were encapsulated in the lungs during breathing and could not be removed by gravity drainage at the termination of liquid breathing. The demonstration of vacuolated macrophages in lungs of dogs following liquid breathing[3] supports such a mechanism. Components of FX-80 would continue to diffuse from such deposits into pulmonary blood, causing a delay in the onset of desaturation and a prolongation of the desaturation process. This

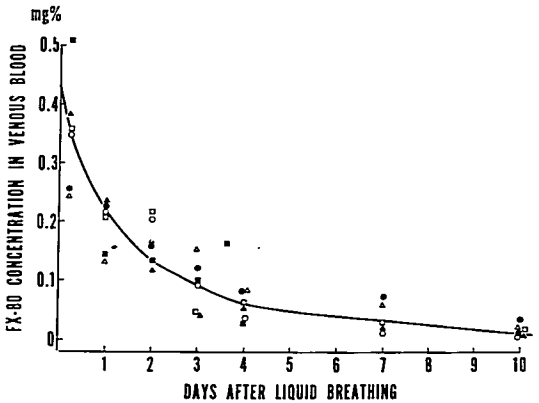

FIG. 5. Desaturation following liquid breathing. The concentrations of FX-80 in the blood of six dogs following liquid breathing are plotted against time. The solid line is the function expressed in equation 2.

TABLE I. Distribution of FX-80 in Blood and Tissues of Dogs

	Group I (1 Dog)	Group II (8 Dogs)	Group III (6 Dogs)
Duration of liquid breathing	8 hours	hour	1 hour
Survival Time	0	3 hours	10 days
FX-80, mg/100 g tissue (mean, SD)			
Blood			
At end of liquid breathing	0.40	0.43 ± 0.095	0.485 ± 0.085
At sacrifice	0.40	0.41 ± 0.097	0.019 ± 0.011
Liver	4.5	2.71 ± 0.78*	0.12 ± 0.11†
Kidney	2.0	4.12 ± 0.79	0.16 ± 0.18
Ovary	4.1	5.57 ± 3.06	0.125 ± 0.008
Muscle	5.2	2.90 ± 0.93	0.62 ± 0.31
Fat	42.0	8.10 ± 5.27	29.5 ± 14.5
Brain			
Gray matter	6.2	4.10 ± 0.45	0.85 ± 0.10
White matter	23.4	8.60 ± 1.72	1.50 ± 0.40
Lung			77.3 (60.8, 93.8)‡

* $n = 7$
† $n = 5$
‡ $n = 2$.
A grossly fatty liver was excluded from each group.

mechanism would also explain why the concentration of FX-80 in adipose tissue of dogs exposed for an hour and allowed to survive for ten days was higher than that of dogs exposed for the same period and sacrificed at three hours.

It is known that a terminal carbon carrying two fluorines loses fluoride rapidly by spontaneous hydrolysis when oxidized.[8] The absence of inorganic fluoride ion in urine in excess of quantities normally present supports the conclusion that fluorocarbon liquid does not undergo biotransformation to any significant extent.

LIPID SOLUBILITY

A high correlation between concentration of lipids and concentration of FX-80 in blood and tissues was found. The concentration of lipids in blood averaged 0.6 per cent and the FX-80 concentration averaged 0.43 mg/100 ml after a steady state was established.

Observed variations in the concentrations of FX-80 and lipids in blood during liquid breathing may have been induced by mobilization of lipid stores caused by periodic injections of heparin.

Since FX-80 is practically insoluble in water, we may presume that essentially all of the FX-80 present in blood is dissolved in lipids. Knowing the partial pressure of FX-80 at the temperature of the liquid in the lungs (50 torr at 35 C), we can calculate a solubility coefficient for FX-80 in blood lipids:

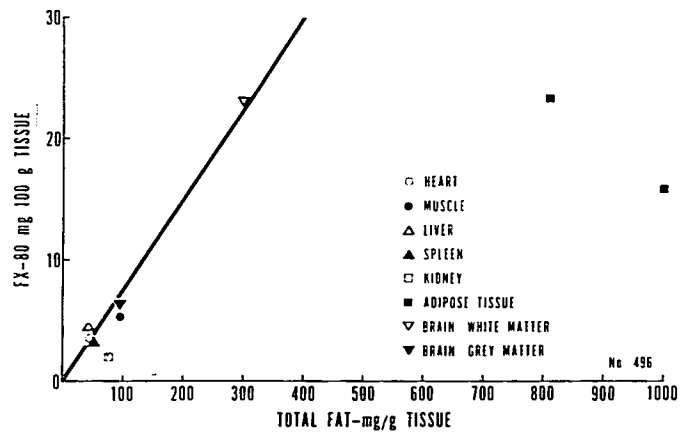

FIG. 6. FX-80 and liquid contents of tissues of a dog in Group I. The dog was sacrificed immediately after eight hours of liquid breathing.

$$\text{Solubility}_{35C} = \frac{0.43 \text{ mg}/100 \text{ ml}}{0.6 \text{ g}/100 \text{ ml}} = 0.717 \text{ mg}$$

FX-80/g lipid/50 torr (3)

Similarly, assuming as a first approximation that FX-80 has the same solubility in all lipids of the body, tissue–blood partition coefficients can be predicted from the ratio of lipid contents of tissues and blood. The concentrations of FX-80 in the tissues during a steady state can then be calculated (table 2).

The observed concentrations of FX-80 in liver, kidney, and reproductive organs (tables 1, 2) were close to the value calculated for the vessel-rich group of tissues (VRG). The calculated values for the muscle group (MG), and especially for the fatty group (FG), were higher than the measured levels, because these tissues could not have reached equilibrium (see equation 6). Only in the dog subjected to liquid breathing for eight hours were the muscles almost saturated.

Kinetics of Uptake

The uptake of drugs by the body can be described as a sum of exponential functions representing the rates of uptake of several tissue compartments.[7] The exponents are rate constants and are functions of the tissue–blood partition coefficients (λ), tissue blood flow (F), and compartmental volume (V). To calculate the rate constants of a lipid-soluble, water-insoluble substance like FX-80, λ can be replaced by the ratio of lipid contents of the tissue and blood (L_{tiss}/L_{bl}):

$$k = \frac{F}{\lambda V} = \frac{F}{V} \cdot \frac{L_{bl}}{L_{tiss}} \quad (4)$$

In order to calculate the rate of whole-body uptake, the coefficients (a_o) are the initial rates of uptake of the compartments and are functions of the rate constants (k), tissue concentration at equilibrium (C), and weight of tissue (W):

$$a_o = kCW = 0.717FL_{bl}D \quad (5)$$

where 0.717 is the solubility in lipid in the case of FX-80, and D is the density of the tissue.

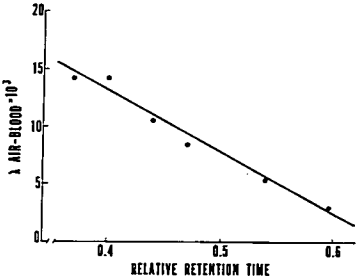

Fig. 7. Correlation between retention times and blood–air partition coefficients. Partition coefficients for individual peaks were estimated by equilibrating blood samples with FX-80 in a tonometer and measuring the heights above baseline of peaks in the air and blood phases. Retention times of individual peaks are relative to that of halothane.

To calculate the a_o's and k's, a three-compartment model was used to represent our dogs (body weight 13.8 kg, cardiac output 2 l/min).[2, 9] Assumed parameters were F_{VRG} 1.5 l/min, F_{MG} 0.35 l/min, F_{FG} 0.12 l/min, V_{VRG} 1.31 l, V_{MG} 519 l, V_{FG} 1.50 l. Values for L_{tiss} and L_{bl} were those given in table 2. Since the whole-body rate of uptake is the product of the difference between the arterial and venous concentrations of FX-80, $(C_a - C_v)$ mg/100 ml, and cardiac output:

$$(C_a - C_v)t = 0.338e^{-0.137t} + 0.74e^{-0.0047t} + 0.22e^{-0.0048t} \quad (6)$$

The differences between concentrations in saturated arterial and venous blood samples obtained from ten dogs during liquid breathing were used as input data for NONLIN,§ a computer program for parameter estimation by successive approximation in nonlinear situations, with a subroutine for the sum of three exponentials. This program, operating an IBM360/65 computer, produced the following equation:

$$y = 0.342e^{-0.136t} + 0.075e^{-0.0047t} + 0.22e^{-0.0005t} \quad (7)$$

§ Made available through the courtesy of Carl M. Metzler of the Upjohn Company.

TABLE 2. Calculated Concentrations of FX-80 in Tissues

Tissue	Lipid Content (g/100 g)	Tissue–Blood λ*	Predicted Concentration of FX-80† (mg/100 g)
Blood	0.6		
Vessel-rich group	5	8.3	3.6
Muscle group	8	13.3	5.7
Fatty group	90	150	64

* $\lambda = \frac{\text{lipid content of tissue}}{\text{lipid content of blood.}}$

† Predicted concentration = lipid content × 0.717 (see equation 3).

with a correlation coefficient, r, of 0.77 for 52 values of $(C_a - C_v)$. Agreement with the theoretical model (equation 6) is striking.

Half-times, τ, predicted by equation 6 are: $\tau_{VRG} = 5.1$ min, $\tau_{MG} = 145$ min, $\tau_{FG} = 1{,}420$ min. Thus, vessel-rich tissues should approach saturation (four half-times) in 20 minutes, muscle in 11 hours, and adipose tissues after four days of liquid breathing. During eight hours of liquid breathing approximately 450 mg of FX-80 should be absorbed by the body. Blood, vessel-rich tissues, and muscle would be, for practical purposes, saturated by eight hours, but adipose tissues should be only 12 per cent saturated because of low blood flow. This explains why the points for adipose tissues in figure 6 fall below the regression line.

The desaturation process was much slower, and the rate constants in equation 2 are much smaller than those predicted by the uptake (equation 7). The high concentrations of FX-80 found in the lungs of dogs surviving the experiment ten days (table 1) support the conclusion that deposits of liquid FX-80 remained in the lungs after liquid breathing, and imply that the concentrations of FX-80 in arterial blood did not approach zero in the desaturation period. Hence, the gradient for washout was smaller than that for uptake.

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