[Chem. Pharm. Bull. 23(6)1368—1373(1975)]

UDC 547.32.08 : 615.31.034.076.9

Excretion of Perfluorochemicals after Intravenous Injection of Their Emulsion

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(Received October 17, 1974)

As a part of studies on perfluorochemicals for candidate as an artificial blood, the elimination of six kinds of the substance was studied to find the way and type of excretion in rats given their emulsions intravenously. (1) These compounds are solely excreted through expiration, none through urinary and biliary with an exception of FC-43 (perfluorotrifbutylamine. (2) The elimination curves of these compounds from the body appear to be exponential). (3) The elimination rate of each substance was fairly related to its vapor pressure, the higher, the more rapidly eliminated. (4) Within six compounds examined sofar, FDC (perfluorodecalin) is selected on account of its rapid elimination.

Since the succeeded experiments of circulatory replacement with the perfluorochemical emulsion reported by Geyer[2,3)] and Clark,[4,5)] many workers have followed them in searching the candidate for artificial blood or a perfusate for preserving isolated organs.[6,7)]

The perfluorochemicals given intravenously in emulsified form have been known to localize in reticuloendothelial system of the liver and the spleen in majority and retained in these organs for a long period of time. Previous studies on its emulsion, however, have been generally limited to the function of oxygen transport, while studies on the fate of the substances in body tissues have not been well established.

The problem of retention of the compounds has been deemed as the major impediment for developing its emulsion as the blood substitute. To solve this problem, it is essential to find a compound which is eliminated from tissues rapidly. For this purpose, we collected the six sorts of perfluorochemical and examined on the elimination of such from the body of animals after intravenous injection of the emulsions.

It has been felt likely, through our past experiments, perfluorochemicals are excreted through expiration and the excretion rate are related to the vapor pressure of each substance. The study reported here has been made to confirm the pathway of excretion of perfluorochemicals after intravenous injection of emulsions and the relation of vapor pressure of each substance to the excretion rate, directing the selection of the compounds as above mentioned.

Experiments

1. Materials——Six kinds of perfluorochemical (Dainippon Ink, Co., Japan) were used in this study. The chemical formulae and important physical constants are listed in Table I. Benzotrifluoride (BTF) was used as an internal standard for gas chromatography. 1,1,2-Trichlorotrifluoroethane (FC-113) and fluorinated polyether (Freon E_4; Dupont chemical, USA) were used for extracting the perfluorochemicals retained in blood and for capturing them in expiration, respectively. These reagents were purified by the method of distillating fractionation before use.

1) Location: *Midorijuji, 1/3, Gamou-cho, Joto-ku, Osaka, 536, Japan.*
2) R.P. Geyer, R.G. Monrow and K. Taylor, *Federation Proc.*, **27**, 384 (1968).
3) R.P. Geyer, *New Engl. J. Med.*, **289**, 1077 (1973).
4) L.C. Clark, F. Becattini and S. Kaplan, *J. Thoracic Cardiovascular Surg.*, **60**, 757 (1970).
5) L.C. Clark, F. Becattini and S. Kaplan, *Triangle*, **11**, 115 (1974).
6) H.A. Sloviter, M. Prtocovic and S. Ogoshi, *J. Appl. Physiol.*, **27**, 666 (1969).
7) I.W. Rosenblum, *Microvascular Res.*, **7**, 307 (1974).

Table I. Chemical and Physical Constants of Perfluorochemicals

	Compounds	Molecular formula and weight	Boiling point	Density at 20° (g/ml)	Vapor press. at 37° (mmHg)
FC-43	perfluorotributylamine	$C_{12}NF_{27}$: 671.13	176—177°	1.87	1.14
FMD	perfluoro-1-methyldecalin	$C_{11}F_{20}$: 512.12	160—161°	1.95	4.8
FDEA	perfluoro-N,N-diethylcyclohexylamine	$C_{10}NF_{21}$: 533.11	148—149°	1.89	8.7
FTC	perfluoro-2-isopentylpyran	$C_{10}OF_{20}$: 528.12	143—144°	1.83	9.9
FDC	perfluorodecalin	$C_{10}F_{18}$: 462.11	142—143°	1.93	12.7
FBA	perfluoro-N-methyldibutylamine	C_9NF_{21}: 521.7	134—136°	1.80	16.0

The abbreviations of the compounds were named by the authors.

2. Preparation of Perfluorochemical Emulsions——Each of the perfluorochemicals was emulsified with yolk phospholipid (supplied by Vitrum AB., Sweden) as follows: the mixture of 150 g of perfluorochemical and 500 ml of 4% phospholipid aqueous suspension was emulsified by passing it 10 times through a Manton-Gaulin homogenizer at the 2nd pressure of 50 kg/cm² and the total pressure of 160 kg/cm² under the nitrogen gas stream. After dissolving sodium chloride to 0.9 w/v%, the emulsion was filtered with a millipore membrane (Nippon millipore Ltd., Japan) of 0.45 micron pore size. The weight average diameter of particles in the emulsions as measured by the centrifugal sedimentation method[8] ranged from 0.1 micron to 0.2 micron.

3. Animal Experiments——Male Wistar strain rats weighing 140 to 160 g were used through all of the experiments. Animals were fed a commercial diet (Nihon Clea Co., Japan). They were injected 4 g/kg body weight of the perfluorochemical through tail vein, and kept in a metabolic cage to collect urine and feces separately. The rats were sacrificed at several intervals after injection and blood was bled by total bleeding at each time prior to sacrifice for determining the retention of the compounds in circulation.

4. Determination of Perfluorochemicals in Blood, Urine and Feces——The method of determination herein used complied with our report[9] as outlined below. One ml of blood was collected into a test tube and then 3 ml of ethanol was added in each to destroy the emulsion. By centrifugation for 10 min at 3000 rpm, the perfluorochemicals were settled down with tissue fragments and supernatant was discarded. Five ml of FC-113 was added to residual precipitation in the tube for extracting of them. After centrifugation again, the lower FC-113 layer was washed twice with water and was dried with anhydrous sodium sulfate. One ml aliquot was transferred into another test tube and 1 to 2 ml of 1.0 v/v% BTF solution in FC-113 was added as the internal standard. Then, this sample was analyzed by gas chromatography. The gas chromatography was done in duplicate. The contents of perfluorochemicals were given from the standard curves which were related to the peak height ratio and the volume ratio of the perfluorochemicals to the internal standard.

The determination of perfluorochemicals in urine and feces was performed by the similar method with blood. The recoveries of these compounds in the blood, urine and feces were 98.6, 97.5 and 95.1%, respectively.

5. Determination of Perfluorochemicals in Expiration——The determination of perfluorochemical excreted through expiration was carried out by using an apparatus shown in Fig. 1. Three absorbance tubes, 30 cm tall and 3 cm i.d., each containing 20 ml of Freon E_4 as the absorbent and 30 ml of water as covering layer were used to capture the perfluorochemicals in expiration. Two rats given the perfluorochemical were placed in a glass vessel (about 10 liters capacity) for 2 or 3 hr at several intervals after the injection and the air was sucked with a suction pump through the exhaust pipe. The air inspired through the inlet pipe was led into the absorbance tubes through the vessel and the vaporized compounds in the expiration were captured in Freon E_4. The air flow was kept between 450 ml and 500 ml per minute during the experiment. One ml of Freon E_4 was collected

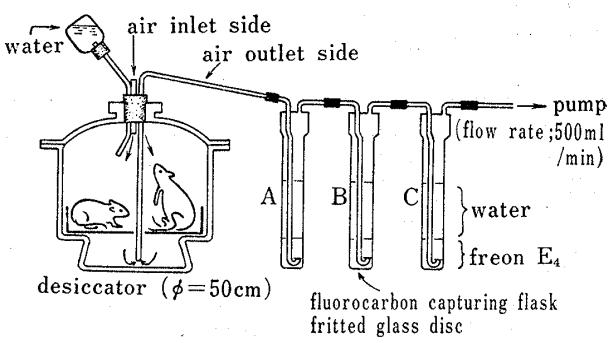

Fig. 1. The Apparatus for Capturing Perfluorochemicals Excreted through Expiration

8) K. Yokoyama, A. Suzuki, I. Utsumi and R. Naito, *Chem. Pharm. Bull.* (Tokyo), 22, 2966 (1974).
9) K. Yamanouchi, R. Murashima and K. Yokoyama, *Chem. Pharm. Bull.* (Tokyo), 23, 1363 (1975).

into a test tube and accurately 1 ml of 1.0 v/v% BTF solution in Freon E_4 was added as internal standard. Then the sample was analyzed by gas chromatography. The capturing efficiencies of FC-43, FMD, FDEA, FDC, FTC and FBA were 99.8, 98.8, 90.5, 93.2, 92.9 and 89.8%, respectively.

6. Gas Chromatography——The gas chromatography was done by the method of our previous report.[9] A Shimadzu Gas chromatograph model GC-4BPTF equipped with flame ionization detectors, was used in this experiment. A column used for determining perfluorochemicals in FC-113 was a coiled glass tube, 2 m long and 4 mm i.d. packed with 20% silicone OV-17 on chromosorb W AW (DMCS), 60 to 80 mesh, and for determining perfluorochemicals in Freon E_4 was a same size tube packed with 15% Carbowax-1540. The two types of analysis were carried out by the same operating condition. The temperature of column oven, injection port and detector were 60, 175 and 180°, respectively. Nitrogen gas flow rate was 40 ml per minute.

Results and Discussion

The expiratory excretion rates of six kinds of perfluorochemical in rats given 4 g of them per kg body weight are shown in Table II. The rates seemed to relate to the vapor pressure of each substance. The excretion rate of FDC which was most rapidly excreted among them was 19.1 mg/hr/kg body weight at 0 to 3 hr after injection and was gradually decreased with time. On the other hand, the rate of FC-43 was only 0.173 mg/hr/kg body weight. No change was found during the first 1 week after the injection.

The blood levels of perfluorochemicals after injection are shown in Fig. 2. At 48 hr after

Table II. Expiratory Excretion Rates (mg/hr/kg body weight) of Perfluorochemicals in Rats Given 4 g per kg Body Weight of the Substance

Perfluoro chemicals	Time after injection					
	3 hr	24 hr	48 hr	96 hr	7 days	14 days
FC-43	0.173±0.021	0.165±0.01	0.158±0.01	0.179±0.03	0.148±0.01	0.159±0.02
FMD	1.34 ±0.16	0.98 ±0.03	0.99 ±0.08	1.03 ±0.04	1.05 ±0.06	0.89 ±0.09
FDEA	2.03 ±0.19	1.75 ±0.20	1.84 ±0.33	1.70 ±0.18	1.62 ±0.04	1.64 ±0.15
FTC	4.01 ±0.31	3.11 ±0.44	3.03 ±0.61	2.99 ±0.43	2.76 ±0.32	2.54 ±0.81
FDC	19.11 ±1.79	13.03 ±0.99	12.60 ±1.41	10.41 ±1.53	8.03 ±0.42	3.20 ±0.61
FBA	5.53 ±0.77	5.17 ±0.70	4.69 ±0.99	4.75 ±0.65	4.48 ±0.79	3.38 ±0.54

Each determined value shows Mean±S.D. obtained from 8 to 10 rats. Values are expressed by mg/hr/kg body weight.

Table III. Amount of Perfluorochemicals Excreted in Urine and Feces in Rats Given 4 g per kg Body Weight

Perfluoro chemicals	Amount of perfluorochemicals excreted (mg/day/kg b.w.)					
	Urine 0—2 weeks	Feces				
		0—1d	1—2d	2—4d	4—7d	7—14d
FC-43	N.D.	1.35	1.18	1.16	2.01	1.17
FMD	N.D.	—	—	N.D.	—	—
FDEA	N.D.	—	—	N.D.	—	—
FTC	N.D.	—	—	N.D.	—	—
FDC	N.D.	—	—	N.D.	—	—
FBA	N.D.	—	—	N.D.	—	—

The data obtained from each 10 rats group.
N.D.: not detected

injection, the retention of FC-43, FMD and FDC in blood was more than 10% of the given dose but that of another was less than 5% and at 1 week, all of them disappeared completely from circulation. This finding indicates that the expiratory excretion rates of these compounds are not related to the blood level of the substances, as these compounds were not detected

in blood at 1 week after injection any more but the expiratory excretion still maintained during first 1 week.

Table III shows the amount of perfluorochemicals excreted through urine and feces of the rats. In urine, no compounds were detected at all. This results are natural, because these compounds are inert, water insoluble and their configuration in the body is only in the form of emulsion.

The perfluorochemicals except FC-43 in feces were not detected. FC-43 content in the feces was less than 0.1% of the given dose through 2 weeks after injection. From these findings, it is obvious that the perfluorochemicals injected intravenously are almost solely excreted through the expiration, not through bile or urine.

Summing the total amount of expiratory excreted compounds through expiration, the retained amounts of the substance in the body were calculated, plotted, on the semilogarithmic graph as shown in Fig. 3. Since the perfluorochemicals are solely excreted through the expiration, the retained amount is only related to the expiratory excretion: the elimination rate indicates the expiratory excretion rate. The elimination curves of these substances appeared to be exponential.

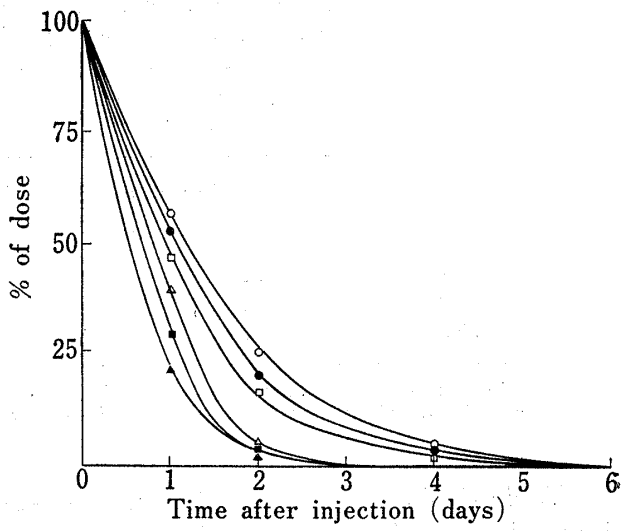

Fig. 2. Elimination Curves of Perfluorochemicals from Circulating Blood of Rats Given 4 g per kg Body Weight of them

Each point represents the average from 8 to 10 rats.
1: FC43 —○—
2: FMD —●—
3: FDEA —△—
4: FTC —▲—
5: FDC —□—
6: FBA —■—

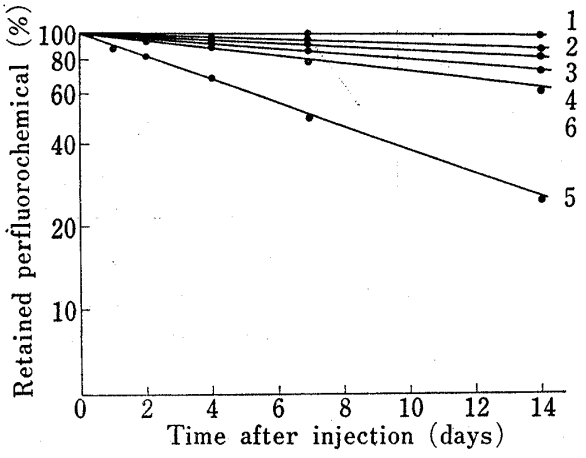

Fig. 3. Elimination Curves of Perfluorochemicals in Rats Given 4 g per kg Body Weight of them in Emulsified Form

The value of retained perfluorochemicals is given by calculating an amount of the expiratory excreted compounds at each day shown in Table II.

1: FC43 3: FDEA 5: FDC
2: FMD 4: FTC 6: FBA

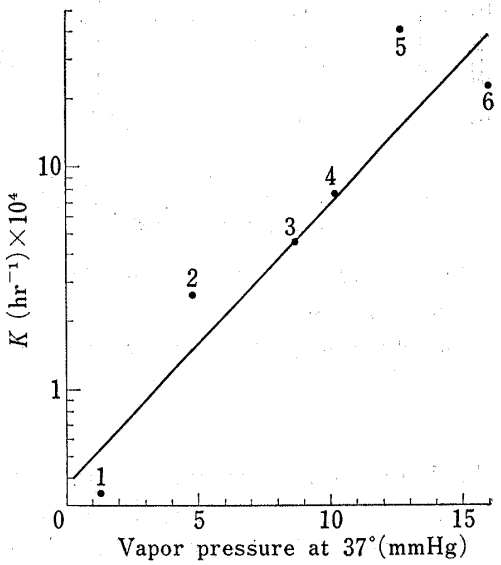

Fig. 4. Relationship between Expiratory Elimination Rate Constant K and Vapor Pressure of Perfluorochemicals

1: FC43 2: FMD 3: FDEA
4: FTC 5: FDC 6: FBA

The relationship between the elimination rate constant K and the vapor pressure of perfluorochemical is shown in Fig. 4. By this figure, it seemed likely that the elimination rate was related to the vapor pressure of each substance in certain extent.

Table IV. Expiratory Elimination Rate Constant K and Consequently Calculated Half-Life Span in Rats given 4 g per kg Body Weight of Perfluorochemicals

Perfluoro chemicals	Vapor pressure at 37°	K	Half-life span
FC-43	1.14 mmHg	0.32×10^{-4} hr^{-1}	895.2 days
FMD	4.8	2.65×10^{-4}	109.0
FDEA	8.7	4.63×10^{-4}	62.4
FTC	9.9	7.55×10^{-4}	38.2
FDC	12.7	40.30×10^{-4}	7.2
FBA	16.0	12.83×10^{-4}	22.5

The expiratory elimination rate from the body was calculated by plotting the retained substances in the body at each time after intravenous injection of emulsions as shown in Fig. 3.

Table IV shows the elimination rate constant and the calculated half-life span of perfluorochemical retained in whole body of rats given 4 g/kg body weight of the substance intravenously. The half-life span of FDC, the most rapidly excreted, was 7.2 days and the retained amount in the body at the 50 days after injection was calculated to be less than 1% of the given dose, while the half-life span of FC-43 was 900 days.

Fig. 5 shows the effect of dose on the elimination rate of FDC in rats given 2, 4 and 8 g

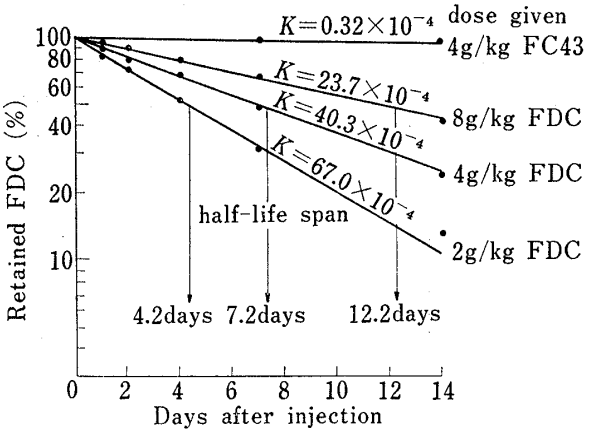

Fig. 5. Effect of Dose on Elimination Rate of FDC in Rats

The value of each point is obtained from 5 to 10 rats.

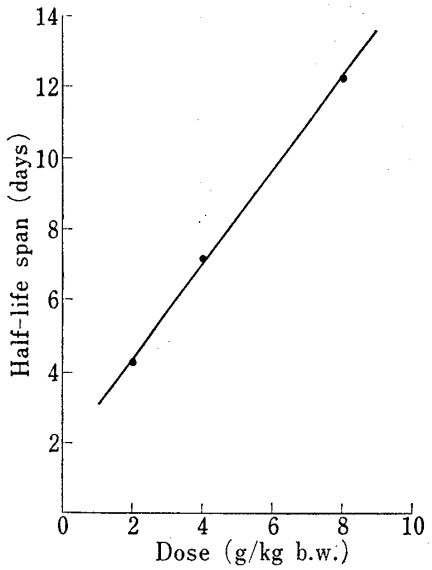

Fig. 6. Relationship between Half-Life Span and Dose of FDC in Rats

per kg body weight intravenously. The rate of elimination related fairly to the amount of FDC given and the each elimination curve seemed also to be exponential.

The relationship between the half-life span and dose of FDC is shown in Fig. 6, showing a good linearity between the given dose of 2 g and 8 g per kg, indicating that the elimination rate constant was dependent on the amount of FDC initially given.

In the present report, we do not discussed on the metabolites of perfluorochemicals. It is generally that the perfluorochemicals are biologically inert and probably not metabolized in the body.[10] In this experiment, the metabolite of perfluorochemicals excreted through expiration have not been detected by the gas chromatographic analysis. However, it is

10) D.A. Holaday, *Federation Proc.*, **29**, 1815 (1970).

necessary to elucidate this problem for developing the perfluorochemicals emulsion as an artificial blood substitute.

It has been agreed that the most important problem in the course of studies on perfluorochemicals as an artificial blood substitute is their retention in the body. This problem may be solved by applying the perfluorochemical having relatively lower boiling point, such as, FDC or FBA. Though there are many other problems to be solved before its. The findings derived from this experiment indicate that a first choice of suitable compounds as a candidate of an artificial blood substitute can be made by their physico-chemical properties, especially the vapor pressure.