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Retention of Perfluorochemicals in Circulating Blood and Organs of Animals after Intravenous Injection of Their Emulsions[1]

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To choose a perfluorochemical as a candidate for the artificial erythrocyte or organ perfusate, screening of perfluorochemicals was performed from the standpoints of persistence of them in the circulation and deposition in the body tissues of rabbits, mice and rats.

In view of elimination from the body, perfluorodecalin was found to be the most suitable compound among five different perfluorochemicals examined. As for emulsifiers, egg yolk phospholipid was found to be the first choice on account of its ability to sustain perfluorochemicals for an adequate period in the circulation as well as the feasibility for preparing emulsion among the three emulsifiers examined. It was found also that the smaller the particle size of emulsion, the better suited for an artificial erythrocyte of perfusate.

Since Geyer, *et al.*[3] and Clark, *et al.*[4] succeeded in retaining animals alive whose blood was totally replaced with perfluorochemical emulsions, the possibility to use these for artificial blood has been studied by many investigators[5] in animals.

It has been well known that perfluorochemicals (FC) are inert *in vivo* and have no pharmacodynamic effects. But the long term retention of the substances in body tissues has been the main impediment for their use as the substitute of blood.

In this study, for selecting the best FC-emulsion for the artificial erythrocyte, the screening of FC was performed from the both viewpoints of the blood level and the degree of retention in the body among five kinds of FC and three kinds of emulsifiers.

Experimental

1. Materials——Five kinds of FCs were used in this study: perfluorotributylamine (FC-43), perfluoro-1-methyldecalin (FMD), perfluorodecalin (FDC) and perfluoro-*N*,*N*-diethylcyclohexylamine (FDEA) supplied by Dainippon Ink and Chemicals Inc., Tokyo, 2H-nonacosafluoro-3,6,9,12-tetraoxa-5,8,11-methylpentadecane (Freon E_4) supplied by Du Pont de nemours & Co., U.S. Within these compounds, FMD and FDC were further purified by distillation before use.

Three sorts of emulsifiers were used in these studies. The commercial polyoxyethylene-polyoxypropylene copolymer (Pluronic F-68) was further purified by ion exchanger "IRA-400" and "IR-120B" resins before use.

Egg yolk phospholipid (YPL) was supplied by AB Vitrum, Stockholm. This consists of 3-*sn*-phosphatidyl cholin in 75% or more as determined by thin-layer chromatography. Perfluorooctane-sulfonyl-amido-dimethylaminopropane-N-oxide (FNNO) was supplied by Dainippon Ink and Chemicals Inc.

2. Preparation of Perfluorochemical Emulsions——Five perfluorochemicals and three emulsifying agents were used for preparing FC-emulsions. Each emulsion consisted of either one of perfluorochemicals in 20% (w/v) FC and either one of surfactants, 4% (w/v) Pluronic F-68, 2% (w/v) YPL or 2% (w/v) FNNO.

1) A part of this paper was presented at the 22nd General Congress of Japan Society of Blood Transfusion held at Osaka, May 1974.
2) Location: *3-5, Miyakojima-nakadori, Miyakojima-ku, Osaka, 534 Japan.*
3) R.P. Geyer, R.G. Monroe, and K. Taylor, *Federation Proc.*, **27**, 384 (1968).
4) L.C. Clark Jr., S. Kaplan, and F. Becatini, *J. Thorac. Card. Surg.*, **60**, 757 (1970).
5) H.A. Sloviter, T. Perkovic, S. Ogoshi, and H. Yamada, *J. Appl. Physiol.*, **27**, 666 (1969); H.A. Sloviter, and T. Kamimoto, *Nature*, **216**, 458 (1967); L. Triner, M. Verosky, D.V. Habif, and D.G. Nahas, *Federation Proc.*, **29**, 1778 (1970).

High pressure with Manton-Gaulin type homogenizer (Manton-Gaulin Mfg., Co., U.S.) was applied for emulsifications.

On each emulsion, weight average diameter of particles in the emulsions was determined by the centrifugal sedimentation method.[6] The diameters were found to range between 0.1 and 0.2 μ. As these emulsions are not isotonic, a certain amount of sodium chloride was added prior to injection to animals.

3. Animals for Experiments——Male rabbits weighing 2.2—3.0 kg were used to trace the elimination of given FC from the circulation. The FC emulsions were administered through ear vein with injection rate of 1.5 ml/min/kg body weight. One to two ml of blood was drawn at various intervals after injection for determining FC level.

Either male dd-strain mice weighing 15—20 g or male Wistar strain rats weighing 200—250 g were used for the study of the distribution of given FC in organ tissues. The animals were sacrificed at various intervals following injection and the FC contents were determined on the homogenates of 0.5—1.0 g of each organ.

4. Determination of FC Content in Blood and Tissues——FC in organs or blood were extracted and determined by the method as described in the authors' previous report.[7]

First, ethanol was added into the homogenates of organs or blood to break the FC-emulsion. The mixture was shaken vigorously and allowed to stand for an hour and the tubes were centrifuged to settle down FC. The upper layers were carefully discarded and a certain volume of 1,1,2-trichlorotrifluoroethane (FC-113) was added to the residual layers in each tube for extracting FC. The tubes were vigorously shaken and centrifuged. The lower layers were washed twice with water. Benzotrifluoride was added as the internal standard accurately into an aliquot of the FC-113 layer. These samples were put to analysis by gas chromatography.

Results and Discussion

1) Influence of the Chemical Structure of FCs on Intravascular Persistence

Five different kinds of FC-emulsions emulsified with YPL were injected intravenously to rabbits with dose of 4 g FC per kg of body weight. Fig. 1 shows the elimination of injected

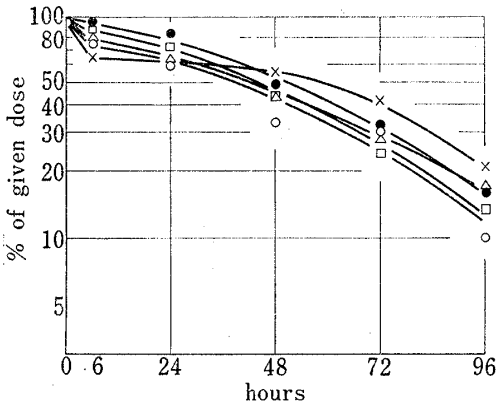

Fig. 1. Elimination of Some Perfluorochemicals, All Emulsified with YPL, from the Circulation in Rabbits

emulsion: 20% (w/v) FC, 4% (w/v) YPL
dose: 4 g/kg *b.w.*
—●—: FC43 —□—: FDC
—△—: Freon E_4 —×—: FDEA
—○—: FMD
Each point represents the mean of at least 3 rabbits.

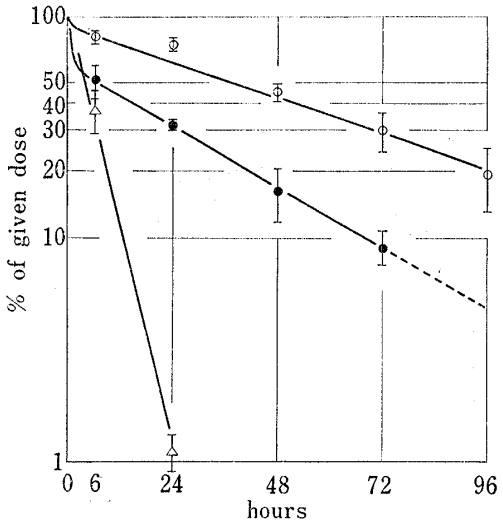

Fig. 2. Elimination of FDC with Different Emulsifiers, YPL, Pluronic F-68 and FNNO, from the Circulation in Rabbits

emulsion: 20% (w/v) FDC
2.0% (w/v) YPL or FNNO, or 4.0% (w/v) Pluronic F-68
dose: 4 g/kg *b.w.*
—○—: YPL
—●—: Pluronic F-68
—△—: FNNO
Results are given as the mean ±S.E. in at least 3 rabbits.

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

FC from blood stream. The levels of FC in blood stream decreased and reached to about 10 to 20% at 96 hours after injection. Although some of FC appeared to decrease rather fast at the early several hours than the subsequent period, the elimination from the circulation of five kinds of FCs emulsified with YPL, however, seemed to have a similar tendency.

2) Influence of the Emulsifiers on Intravascular Persistence of FCs

Three kinds of emulsifiers, YPL, Pluronic F-68 and FNNO, were used for preparing of FDC emulsion.

The elimination half life spans of each FDC emulsified with YPL, Pluronic F-68 and FNNO from the circulation in rabbits given 12 g of FDC per kg of body weight were approximately 44, 26 and 4 hours respectively as shown in Fig. 2.

FDC emulsified with FNNO disappeared rapidly from the circulation, while the same emulsified with YPL decreased slowly.

Similar results were observed in FC-43 emulsions prepared with each of three emulsifiers by another experiment.

The difference in the elimination rate among three FDC emulsions with different emulsifiers may assumably be resulted from different stability of each emulsion in the circulation.

3) Influence of the Particle Size of Emulsion on Intravascular Persistence

To examine the influences of the particle size of emulsion on the intravascular persistence of FC, "fine" emulsion consisting mainly of smaller particles (the average diameter of particles: 0.095 μ) and "coarse" emulsion of larger particles (the average diameter of particles: 0.3 μ) were injected intravenously to rabbits with the dose of 12 g FC per kg of body weight.

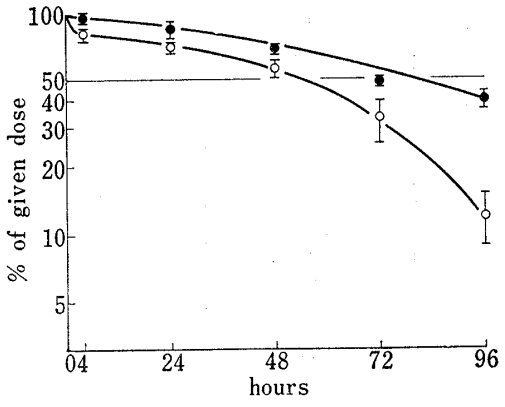

Fig. 3. Comparison of "Fine" and "Coarse" Emulsion on Eliminations of FC from the Circulation in Rabbits

emulsion: 20% (w/v) FC43, 4.0% (w/v) Pluronic F-68
dose: 12 g/kg *b.w.*
—●—: "fine" emulsion (the average diameter of particles: 0.095 μ)
—○—: "coarse" emulsion (the average diameter of particles: 0.3 μ)
Results are expressed as the mean±S.E. in at least 3 rabbits.

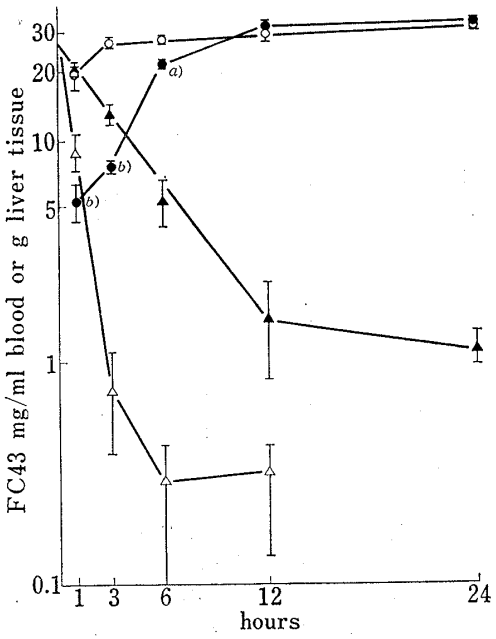

Fig. 4. Blood and Liver Levels of Rats Given "Fine" and "Coarse" Emuslion of FC

emulsion: 20% (w/v) FC43, 4.0% (w/v) Pluronic F-68
dose: 2 g/kg *b.w.*
—○—: "coarse" emulsion } FC 43 mg/g liver tissue
—●—: "fine" emulsion }
—△—: "coarse" emulsion } FC43 mg/ml blood
—▲—: "fine" emulsion }
Results are given as the mean ±S.E. in at least 5 rats.
Significant differences from rats given "coarse" emulsion are marked: *a*) ($p < 0.05$), *b*) ($p < 0.01$)

As shown in Fig. 3, FC-43 level in blood of the rabbits given "coarse" emulsion decreased more rapidly than that given "fine" at the begining and later decreased gradually along the same rate as that given "fine" until 72 hours.

Similar results were obtained in both Freon E_4 and FMD emulsions prepared with YPL.

In order to clarify these phenomena, the relation between the elimination of FC from the circulation and the retention in liver was studied comparing "fine" with "coarse" emulsion. As shown in Fig. 4, in the rats given "coarse" FC-43 emulsion, the blood level of FC-43 decreased rapidly and its retention in liver increased intensely for early several hours after injection, while in the rats given "fine" emulsion, the elimination from blood was slower than that of "coarse" and the retention in the liver was significantly smaller.

These results indicate that the particle size influenced on the deposition of FC particles into the reticuloendothelial system (RES). This is also supported by the report of Hayashi[8] that the "coarse" FC-emulsion effects more intensively inhibition of RES-activity at early stage after injection than the "fine" one.

4) Distribution of FC in Organ Tissues

Table I shows the content of FC in various tissues of rabbits at one week after intravenous injection of 12 g per kg of body weight. FC was mostly deposited in the liver, spleen and

TABLE I. Distribution of FC in Organs of Rabbits at a Week after Injection (Dose; 12 g/kg b.w.)

	FC43/Pluronic F-68		Freon E_4/YPL		FMD/Pluronic F-68	
	mg/g (fresh tissue)	% of dose	mg/g (fresh tissue)	% of dose	mg/g (fresh tissue)	% of dose
Brain	N.D.[a]		N.D.		N.D.	
Heart	2.9± 1.4[b]		0.8± 0.3		N.D.	
Lung	65.8± 21.6	3.2± 1.4	145.1± 38.9	6.2± 2.1	30.7± 7.8	1.9±0.4
Liver	134.4± 56.2	45.8±13.4	96.7± 17.6	35.5±12.1	36.5±11.5	30.8±9.8
Spleen	347.7±105.6	4.4± 1.8	351.7±131.8	14.7± 5.2	242.1±58.4	9.3±2.4
Kidney	6.6± 3.2	0.3± 0.1	9.2± 3.6	0.5± 0.2	1.1± 0.3	0.2±0.07
Adrenal	51.3± 21.6		6.8± 4.1		N.D.	
Pancreas	25.4± 8.5		2.0± 0.5		N.D.	
Small intestine	6.3± 2.8		trace		N.D.	
Stomach	0.6± 0.2		0.1± 0.05		trace	
Colon	5.1± 2.1		2.6± 1.2		0.5± 0.2	
Femoral marrow	120.2± 48.6		73.2± 26.9		32.4± 9.2	
Muscle	0.1± 0.02		trace		N.D.	
Adipose tissue	0.1± 0.02		0.2± 0.05		N.D.	
Bile	N.D.		trace		N.D.	
Eye ball	0.1± 0.04		N.D.		N.D.	

a) N.D.: not detected.
b) The data are presented as the mean ± S.E. The number of rabbits is at least 5 in each group.

lung. Fourty % or more of given dose were found to be deposited in these organs. The highest concentration of FC, however, was found in the spleen and fairly high content was in the femoral marrow and liver.

The content of FMD in the body seemed to be somewhat less than those of Freon E_4 and FC-43. However, no significant difference was found in the tissue level among three kinds of FCs at one week after injection.

To investigate the changes of the retention of FC in the body for a longer period following injection, the contents of three FCs in main organs such as the liver, spleen, lung and kidney were determined with mice receiving either one of three kinds of FC-emulsions at various

8) G. Hayashi (Tanabe Seiyaku Co., Ltd.), private commiuncation.

Table II. Changes of FC Content in the Liver, Spleen, Lung, and Kidney of Mice Given Each of Three Different FC-Emulsions intravenously (Dose: 4 g/kg b.w.)

Time after injection	Tissues	FC43/YPL	Freon E_4/YPL	FMD/YPL
48hr	liver	48.8 ± 9.6[a]	51.4 ± 8.5	48.4 ± 1.6
	spleen	8.2 ± 0.48	17.3 ± 8.8	1.4 ± 0.63
	lung	0.12± 0.10	0.50± 0.02	trace
	kidney	1.9 ± 0.64	1.0 ± 0.62	trace
	total	59.1	70.2	49.8
1 week	liver	35.4 ±12.1	52.2 ±12.3	48.9 ± 7.9
	spleen	8.0 ± 6.1	12.2 ± 6.1	4.5 ± 1.4
	lung	0.03± 0.01	trace	trace
	kidney	trace	0.54± 0.11	trace
	total	43.4	64.7	53.4
2 weeks	liver	30.2 ±14.5	54.0 ±15.8	28.4 ± 7.3
	spleen	3.9 ± 2.4	1.7 ± 0.57	4.1 ± 0.81
	lung	trace	trace	trace
	kidney	trace	0.53± 0.01	trace
	total	34.1	56.2	32.5
4 weeks	liver	35.9 ± 6.1	53.5 ± 7.2	20.8 ± 4.1
	spleen	3.8 ± 0.46	6.9 ± 3.2	2.4 ± 0.64
	lung	0.06± 0.03	trace	trace
	kidney	0.73± 0.09	0.10± 0.05	trace
	total	40.5	60.5	23.6
8 weeks	liver	37.3 ± 4.7	64.1 ± 9.1	5.1 ± 2.1
	spleen	2.9 ± 1.6	6.4 ± 3.1	1.1 ± 0.30
	lung	trace	0.14± 0.02	trace
	kidney	0.52± 0.30	0.40± 0.09	trace
	total	40.8	71.0	6.2

a) Figures mean percent of given dose and express as the mean ± S.E. in at least 4 mice.

Table III. Changes of FC Content in the Liver, Spleen, and Lung of Rats Given FMD or FDC-Emulsion intravenously (Dose: 8 g/kg b. w.)

Time after injection	Tissues	FMD/YPL	FDC/YPL
1 week	liver	37.6 ± 5.5[a]	18.3 ± 3.1
	spleen	14.8 ± 2.8	7.5 ± 3.7
	lung	0.13 ± 0.06	trace
	total	52.5	25.8
2 weeks	liver	31.4 ± 5.5	3.8 ± 1.2
	spleen	23.8 ± 2.0	4.8 ± 2.3
	lung	0.05 ± 0.02	N.D.[b]
	total	55.2	8.6
4 weeks	liver	20.6 ± 2.2	trace
	spleen	10.1 ± 3.2	0.13 ± 0.03
	lung	0.03 ± 0.01	N.D.
	total	30.7	0.13

a) Figures mean percent of given dose and express as the mean ± S.E. in at least 5 rats.
b) N.D.: not detected

intervals until 8 weeks after injection. The results are shown in Table II. The FC injected was mostly deposited in the liver and spleen. Mice given FC-43 retained such high content of FC in the body as 40% of given dose from one week to 8 weeks after injection. Animals given Freon E_4 also retained its high level of 60—70% of the given dose until 8 weeks.

On the contrary, the amount of FMD deposited in those organs decreased most rapidly among these FCs. To confirm the result, a similar experiment was repeated in rats given 8 g per kg of body weight intravenously in comparing FMD and FDC, whose chemical structures resemble each other. The results are summerized in Table III. As the result, it was found that FDC was eliminated more rapidly than FMD from the body.

As for the way of excretion of FCs, the authers' previous report demonstrated that the compounds were mostly excreted through the expiration.[9]

Regardless of the sort of FCs, the elimination rate of FC from the circulation was almost alike each other at a same dose level of FC (Fig. 1) and FC emulsified with YPL was the most stable in blood stream (Fig. 2). In addition, the smaller the particle size of FC emulsion, the less affected the RES-activity. Finally, on the elimination rate of FC from the body which is one of the important probrems to be solved, the elimination rate of FDC was the most rapid among the five different FCs examined. From the results described above, it is concluded that FDC/YPL emulsion with fine particle size may be the selected for the candidate of the artificial blood or perfusate for isolated organs.

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9) K. Yokoyama, K. Yamanouchi, and R. Murashima, *Chem. Pharm. Bull.* (Tokyo), **23**, 1368 (1975).