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Role of Oncotic Agents in Saving Effect of Perflurochemical Emulsions in Hemodilution

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Survival prolongation was studied in rats which were hemodiluted to a hematocrit value of as low as 7, 4, or 1% by stepwise exchange transfusion, alternating bleeding through the artery and infusing a perfluorochemical (FC) emulsion into the vein, with the combined use of oncotic agents taken into consideration to maintain the circulating plasma volume, and the following results were found:

(1) Plasma expander (PE) was indispensable to the use of FC emulsion for the survival of exchange-transfused rats. As an oncotic agent to be combined with FC emulsion, homologous plasma and 6% hydroxyethyl-starch (HES) solution were better than dextran in rats.

(2) At the exchange transfusion with FC emulsion+PE, totaling 1.2 times the volume of blood eliminated, survival effect was better at a ratio of FC emulsion to PE of 1 : 3 than 3 : 1.

(3) Supplementary infusions of Fluosol-DA (an improved FC emulsion made isooncotic with 2.7% HES) carried out after the exchange transfusion at intervals of 24 hr were effective in prolongation of survival.

Keywords——perfluorochemical emulsion; artificial blood; perfluorotributylamine; perfluorodecalin; perfluorodibutylmethylamine; Pluronic F-68

Because of the high solubility of perfluorochemicals (FCs) for oxygen, the possibility of their substituting for red cells has been investigated.[2] Among these chemicals, perfluorotributylamine (FC-43), perfluorodecalin (FDC), and perfluorodibutylmethylamine (FBA) have been selected as candidates for artificial blood materials on account of feasibility for emulsification and low toxicity,[3] but there are still many problems to be solved.

When the FC emulsion is considered to be a part of artificial blood, other functions of the blood, such as oncotic pressure provided by plasma protein and osmotic pressure by electrolytes in plasma, have to be furnished.[4] Plasma expanders (PEs) such as dextran and hydroxyethyl-starch (HES), and electrolytes as in Ringer solution should be combined, as Geyer[2d,3] emphasized in the very early stage of his research.

This report summarizes our studies to clarify how these oncotic agents work and how these should be used to achieve the aimed effect.

Experimental

Materials——Perfluorotributylamine (FC-43), perfluorodecalin (FDC), and perfluorodibutylmethylamine (FBA), synthesized and purified by distillating fractionation at Dainippon Ink, Co., Tokyo were emulsified

1) Location: *1-47, Chuoh 1-chome, Joto-ku, Osaka 534, Japan.*

2) *a*) L.C. Clark, Jr. and F. Gollan, *Science*, **152**, 1755 (1966); *b*) F. Gollan and L.C. Clark, Jr., *Physiologist*, **9**, 191 (1966); *c*) H.A. Sloviter and T. Kamimoto, *Nature* (London), **216**, 458 (1967); *d*) R.P. Geyer, R.G. Monroe, and K. Taylor, *Fed. Proc.* **27**, 384 (1968).

3) *a*) R.P. Geyer, R.G. Monroe, and K. Taylor, "Organ Perfusion and Preservation," Appleton Century Crofts, New York, 1968, pp. 85—96; *b*) L.C. Clark, F. Becattini, and S. Kaplan, *Triangle*, **11**, 115 (1972).

4) R.P. Geyer, *New Engl. J. Med.*, **289**, 1077 (1973); *idem, Bull. Parenteral Drug Assoc.*, **28**, 88 (1974).

with Pluronic F68 (poly(oxyethylene)-poly(oxypropylene) block copolymer), egg yolk phospholipid, and Pluronic F68 and egg yolk phospholipid for FC-43, FDC, and FDC-FBA mixture to prepare Fluosol-43, Fluosol-DC, and Fluosol-DA, respectively.

Constituents of these emulsions are summarized in Table I. Manton-Gaulin homogenizer (Manton-Gaulin Co., U.S.A.) was used for emulsification. Constant care was taken to make fine emulsions with weight average particle size as fine as 0.1 μm or smaller and particles larger than 0.4 μm were excluded. These emulsions were mixed with one of pooled heparinized plasma of rats, 6% HES in Ringer lactate solution, and 6% Dextran 40 in Ringer lactate solution just before use. Either lactated Ringer or Krebs-Ringer bicarbonate buffer solution was used as an electrolyte solution. Animals used were exclusively Wistar strain male rats, weighing 200—240 g.

Table I. Constituents of Emulsions (w/v %)

Constituent		FC emulsion		
		"Fluosol-43"	"Flusol-DC"	"Flusol-DA"
FC	FC-43	35.0	—	—
	FDC[a]	—	28.0	19.6
	FBA[b]	—	—	8.4
Surfactant	Pluronic-F68	4.0	—	3.4
	phospholipid	—	4.0	0.6
	K-oleate	—	0.02	0.004
	Glycerol	—	—	1.0
	D-sorbitol	—	3.9	—

a) Perfluorodecalin.
b) Perfluorodibutylmethylamine.

Procedure for Exchange Transfusion (Hemodilution)——After 12 hr of starvation and under narcosis with sodium thiopental (37.5 mg/kg body weight), rats were fixed in the supine position, catheterized with a polyethylene tube into the carotid artery and a polyethylene tube needle into the tail vein, and kept in an oxygen chamber with standard gas (95% O_2 and 5% CO_2, Po_2 controlled within 600—700 mmHg). Bleeding at the rate of 5 ml/kg body weight/min through the carotid artery followed by replacement with 5—6 ml infusion/kg body weight/min through the tail vein were repeated until the hematocrit reached either 7, 4, or 1%. After removal of the catheters, rats hemodiluted to the desired hematocrit value were transferred to an oxygen chamber with Po_2 of 500—600 mmHg, and fed freely with feed and water. Oxygen content in the arterial blood of rats was measured with LEX-O_2-CON TN (Lexington Instruments Co., U.S.A.). With some of the rats, Po_2, Pco_2, pH of circulating blood, and blood and plasma volume were measured with the IL meter (IL 113-sl, U.S.A.) and ^{125}I-RISA (Auto Gamma-counter, Packard Tandem 5025, U.S.A.) at the completion of the desired hemodilution. Blood level of FC substances was determined by gas chromatography (Shimadzu GC4BPTF, Kyoto).

Results

Preliminary Selection of PEs to be Combined

The emulsion used here was FDC/phospholipid, made isotonic by the addition of Ringer-lactate constituents. The three PEs, homologous pooled plasma of a rat, 6% HES in Ringer

Table II. Survival Hours and Blood Gas Analysis (Rat, Hct=7)

Group	PaO_2	$PaCO_2$	pH	Survival hours
FDCe+L.R.	375	44.5	7.18	0:15
FDCe+Dex. 40	398	32.0	7.33	1:25[a]
FDCe+HES	412	38.0	7.36	5:25[b]
FDCe+Plasma	408	38.5	7.45	12:42[b]
L.R.	358	38.0	7.13	0:06

Ratio of mixing the FDC emulsion and the plasma-expander was 3 : 1.
a) Significant difference from FDC+L.R group ($p<0.05$).
b) Significant difference from FDC+L.R group ($p<0.01$).

lactate, and 6% Dextran 40 in Ringer lactate were compared, with Ringer lactate as a control. Ratio of mixing the FDC emulsion and PE was 3: 1. Exchange transfusion was repeated with FDC emulsion/PE mixture 1.2 times the shed volume. After 15 times or so of repetition, hemodilution reached 7±0.5% hematocrit.

Po_2, Pco_2, and pH of blood, and hours of survival after completion of hemodilution (Hct=7%) are shown in Table II.

As shown in Table II, the combined use of the PEs enhanced survival, preventing acidosis and their effect decreased in the order of pooled plasma, HES, and Dextran 40 (As is well known, rats are sensitive to dextran, and dextran accelerates precipitation of FDC/phospholipid emulsion more strongly than HES.). It can be safely concluded that the combined use of PE, either plasma or HES, is indispensable to the use of FDC emulsion.

Selection of Three FC Emulsions under Different Ratio Combination

In this experiment, effect of FC emulsion/PE mixture was studied by comparing (1) three kinds of emulsions (Fluosol-DA, Fluosol-DC, and Fluosol-43), (2) two kinds of PEs (pooled rat plasma and 6% HES in Ringer lactate), and (3) different ratio of FC emulsion/PE mixture (3: 1, 1: 1, 1: 3), at different rate of hemodilution (Hct=7, 4, 1%). The concentrations of FCs were 32% (w/v) in Fluosol-43, 25% (w/v) in Fluosol-DC, and 25% (w/v) in Fluosol-DA, all not containing PE.

The technique of hemodilution was the same as mentioned above. Survival of rats in a glass oxygen chamber was observed (Fig. 1). In the group with hemodilution of Hct=7%, rats receiving no FCs but PEs survived longer than 72 hr. The effect of PEs was apparent in the groups of rats receiving Ringer lactate/PE mixture indicating that rats of Hct=7% can survive when only a sufficient volume of iso-oncotic PE is given.

In the groups with hemodilution of Hct=4%, prolongation of survival was marked in the groups receiving Fluosol-43 or Fluosol-DA, but not in the groups given Fluosol-DC, while rats receiving only PE+Ringer lactate died within 12 hr. The best results were found in the group which received a mixture of 1 part of Fluosol-43 or Fluosol-DA and 3 parts of PE.

In the case of Hct=1%, most remarkable effect of Fluosol-43 and Fluosol-DA was seen in the groups receiving Fluosols+HES (1: 3) mixture, which resulted in 19—20 hr survival while the control group not receiving FCs survived only for 30 min or shorter.

It could be safely concluded from these finding that the effect of FC-43 and FDC+FBA emulsions on the survival is more apparent in severely hemodiluted (Hct=1%) animals when a sufficient volume of PE (6% HES) is combined. This fact indicates the importance of maintaining the plasma volume besides the oxygen supplying effect of FCs.

Fluosol-DC (FDC/phospholipid emulsion) gives poorer effect, in agreement with its unstability in circulation.[5]

Effect of FC Emulsion/PE Mixture on Circulating Blood and Plasma Volume, Plasma Protein Level and Other Physiological Parameters, and Transition of Blood Level of FCs

In order to understand and clarify the physiological background of the results shown in Fig. 1, the following experiments were carried out.

Blood Volume after Exchange Transfusion with FC Emulsion/PE Mixture——Circulating blood volume was measured by means of ^{125}I-RISA when the hemodilution reached Hct=7% after repeated exchange transfusion with a mixture of Fluosol-DC and PEs at different ratios of mixing. Red cell volume was calculated from the hematocrit values.

As shown in Fig. 2, circulating blood volume was larger in proportion to larger ratio of plasma or HES to FDC emulsion. The trend was more remarked in the group given pooled plasma than of HES.

5) H. Okamoto, M. Iwai, Y. Tsuda, and K. Yokoyama, *Proc. Symp. X Int. Congr. Nutr.*, **1975**, 73.

Hct	FCe. \ pl. exp. / FC : pl. exp.	Homologous plasma 3 : 1	Homologous plasma 1 : 1	Homologous plasma 1 : 3	HES in L.R. 3 : 1	HES in L.R. 1 : 1	HES in L.R. 1 : 3
7	F-DA	45 : 40	36 : 35	42 : 18	46 : 00	55 : 15	72
	F-DC	12 : 40	15 : 50	22 : 10	5 : 25	6 : 40	5 : 53
	F-43	25 : 00	50 : 00	72	6 : 20	72	72
	Control L.R.	3 : 50	28 : 10	72	4 : 45	26 : 50	72
4	F-DA	31 : 00[a]	27 : 54[a]	50 : 00[a]	22 : 13[a]	39 : 25[a]	61[a]
	F-DC	3 : 10	11 : 37	11 : 05	0 : 18	3 : 08	5 : 02
	F-43	18 : 22[a]	11 : 30[b]	29 : 02[a]	7 : 05[a]	30 : 15[a]	57 : 40[a]
	Control L.R.	1 : 00	6 : 40	10 : 30	1 : 28	1 : 45	3 : 30
1	F-DA	6 : 40[a]	10 : 30[a]	15 : 50[a]	6 : 30[b]	11 : 05[a]	19 : 55[a]
	F-DC	0 : 34	5 : 55[b]	8 : 10[b]	0 : 20	2 : 56	2 : 58
	F-43	9 : 42[a]	13 : 28[a]	2 : 50[b]	4 : 00[b]	11 : 20[a]	19 : 37[a]
	Control L.R.	0 : 12	0 : 39	0 : 39	0 : 10	0 : 27	0 : 27

a) $p<0.01$ b) $p<0.05$

24hr

Fig. 1. Survival Hours after Exch.-transfusion ($n=5$)

Under the same level of hematocrit (=7%), the total red cell mass was larger when the total blood volume was larger and this may help to understand the result shown in Fig. 1 that the increased ratio of PEs gave a better survival of the animals. The importance of restoring circulating blood volume is substantiated here.

Transition of FC Level in Blood during Repeated Exchange Transfusion——Level of FCs was measured by means of gas chromatography with blood of rats given repeated exchange transfusion with Fluosol-DC/plasma mixture. As shown in Fig. 3, FDC level increases by repetition of exchange transfusion and is higher in parallel with higher ratio of the FDC emulsion to plasma.

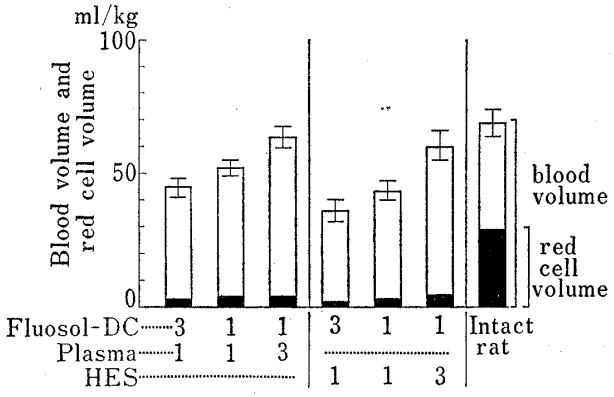


Fig. 2. Circulating Blood Volume and Red Blood Cell Volume after Exchange-transfusion when Hct=7

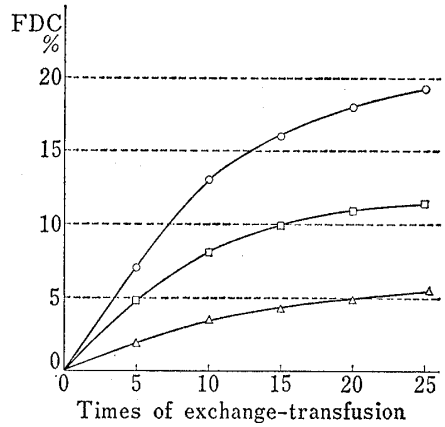


Fig. 3. Concentration of FDC (Rat)

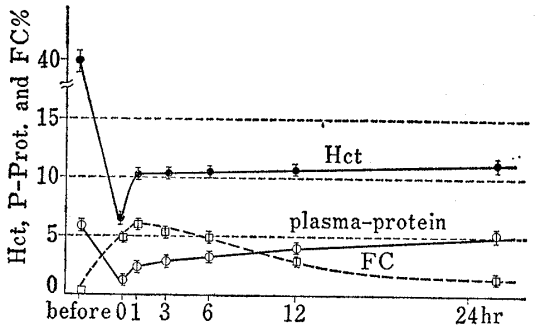
○—○, Fluosol-DC : plasma=3 : 1.
□—□, Fluosol-DC : plasma=1 : 1.
△—△, Fluosol-DC : plasma=1 : 3.

The relation between the repetition of exchange transfusion in compliance with the method described above and the resultant hematocrit is known as follows: 20 repetitions of exchange, 4%; 25 repetitions, 1—2%.[6]

Referring to Fig. 1, the result in Fig. 3 indicates that when the concentration of FDC in blood is as low as 5% (w/v), it is capable of prolonging survival of rats in the state of acute red cell dificiency as Hct=1—2%.

Transition of Circulating Blood and Plasma Volume, Hematocrit, Plasma Protein, and Level of FC Substance in Bood after Completion of Exchange Transfusion——After completion of the repeated exchange transfusion with a mixture of Fluosol-DA+6% HES (1: 3) until hematocrit reached 7%, transition of the concentration of FC substances (FDC+FBA) in blood, circulating blood and plasma volume, plasma protein level, and hematocrit were examined and its results are shown in Fig. 4 and 5.

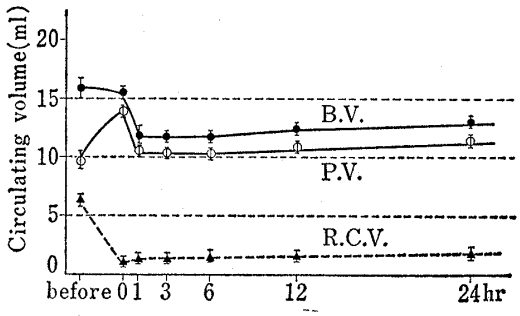
As shown in Fig. 4, the hematocrit value was rapidly restored within 1 hr after completion of the exchange transfusion. This was found to be inversely proportional to the decrease of circulating blood volume. The hematocrit value and plasma protein level were



Hours after Exch.-transfusion with Eluosol-DA(1v.) and 6% HES (3v.), Fnal Hct=7.

Fig. 4. Hematocrit, FC Contents and Plasma-protein after Exch.-transfusion

standard deviation.



Hours after Exch.-transfusion with Fluosol-DA(1v.) and 6% HES(3v.), Final Hct=7.

Fig. 5. Blood Volume, Plasma Volume and Red Cell Volume after Exch. transfusion

standard deviation.

6) K. Yokoyama, K. Yamanouchi, M. Watanabe, T. Matsumoto, R. Daimoto, T. Hamano, H. Okamoto, T. Suyama, R. Watanabe, and R. Naito, *Fed. Proc.*, **34**, 1978 (1975).

then gradually restored, especially the latter reached normal value after 24 hr. The concentration of FC in blood decreased slowly to one-half at 12 hr. No significant change of pH in arterial and venous blood was seen during the 24 hr. SGOT and SGPT were proportional to the dilution of blood and returned to normal after 12 hr.

Effect of Supplementary Infusion with FC Emulsion after Exchange Transfusion

Twelve hr after completion of the exchange transfusion with a mixture of Fluosol-DA emulsion+6% HES in Ringer lactate (1: 3) until Hct=1%, Fluosol-DA was given to supplement eliminated fluorocarbon at the rate of 25 ml/kg body weight.

Table III. Effect of Re-infusion after 12 hr on Surivival

	Re-infusion after Exch.-transfusion	Survival hours after Exch.-transfusion
F.DA : HES=1 : 3	Fluosol-DA 25 ml/kg	45:42±4:23
Final Hct=1	Ringer lact. 25 ml/kg	20:20±1:10
	None	19:55±1:05

As shown in Table III, survival of rats for 20 hr without re-infusion or with Ringer lactate solution only, was prolonged to 45 hr by the re-infusion of Fluosol-DA 12 hr after completion of the exchange transfusion.

During this course of studies, the composition of Fluosol-DA was rearranged and its constituents after mixing before use were as follows (all in per cent (w/v)): FDC 16.00, FBA 6.40, Pluronic F68 2.72, egg yolk phospholipids 0.40, glycerol 0.80, and HES 2.70 in the medium of Krebs Ringer bicarbonate (NaCl 0.480, KCl 0.027, $CaCl_2$ 0.022, $MgCl_2$ 0.016, $NaHCO_3$ 0.168, and glucose 0.144; osmolarity 336 mosm, pH 7.4—7.6, oncotic pressure 380 mmH_2O.

Since replacement for the lost blood volume either with blood or PE should not be neglected as a factor to achieve a good effect, and the new composition of Fluosol-DA is made iso-oncotic with 2.7% HES, the following preparatory experiment was done to clarify the adequate volume of replacement at the exchange transfusion with the FC emulsion.

At each exchange transfusion, as described above, various volumes of the new composition to make each replacement volume 0.8, 1.0, 1.2, and 1.4 times the lost volume were infused until hematocrit reached 7% and survival was observed.

Table IV. Exch.-transfusion with Fluosol-DA[a] (Volume Effect)

Transfusion volume (×shed volume)	×0.8	×10	×1.2	×1.4
Survival hours (Hct=7)	47:05±4:10	53:18±4:21	51:32±3:14	22:00±6:35

a) Iso-oncotic emulsion.

Table V. Exch.-transfusion with Fluosol-DA and the Effect of Re-infusion repeated

Exch.-transfusion with	Re-infusion after Exch.-transfusion		Survival hours (Hct=4)
	24 hr	48 hr	
Fluosol-DA[a]	Fluosol-DA[a] 20 ml/kg	Fluosol-DA[a] 20 ml/kg	65:40±6:42
Fluosol-DA[a]	Fluosol-DA[a] 20 ml/kg	None	55:44±5:30
Fluosol-DA[a]	6% HES	None	48:38±5:08
6% HES	None	None	3:36±0:42

a) Iso-oncotic emulsion.

As shown in Table IV, the adequate volume of replacement in the exchange transfusion was found to be 1.0x (equal with) the bled volume.

Supplementary infusion was given with the new composition or 6% HES in Ringer lactate 24 hr after completion of the exchange transfusion to Hct=4% with the new composition, each time with the same volume as the lost blood. In one group the new composition was given again 48 hr after the exchange transfusion.

As shown in Table V, additional infusion of the new composition 24 hr after completion of the exchange transfusion (Hct=4%) prolonged the survival more than that of 6% HES solution. The second supplementation resulted in 10 hr prolongation of survival.

Optimum Concentration of the FCs in Fluosol-DA

Despite the finding mentioned above that the mixture of 1 part Fluosol-DA and 3 parts PE gave a better survival than mixtures of 1: 1 or 3: 1, the dose of the FCs in the former may be insufficient for oxygen supply due to dilution with the PEs and more of the FC substance could be given with better effect in combination with oncotic agents.

Table VI. Constituents of Fluosol-DA and Fluosol-43

Fluosol	FC conc.[a] (%)		HES conc.[a] (%)
Fluosol-43	28	+	2.6
(oncotic pressure 380—395	25	+	2.7
mmH_2O)	20	+	2.9
	15	+	3.3
	10	+	3.5
	5	+	3.7
Fluosol-DA	22	+	2.7
(oncotic pressure 385—395	20	+	2.9
mmH_2O)	15	+	3.3
	10	+	3.5
	5	+	3.8
Control	0	+	4.0

a) In Krebs Ringer bicarbonate buffer solution.

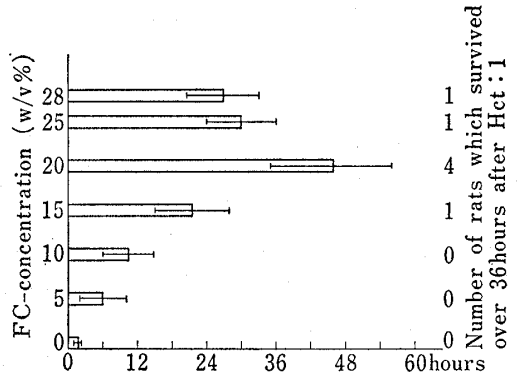


Fig. 6. Survival Hours of Rats treated with Fluosol-43

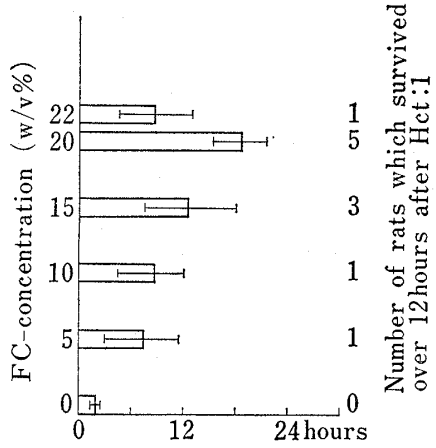


Fig. 7. Survival Hours of Rats treated with Fluosol-DA

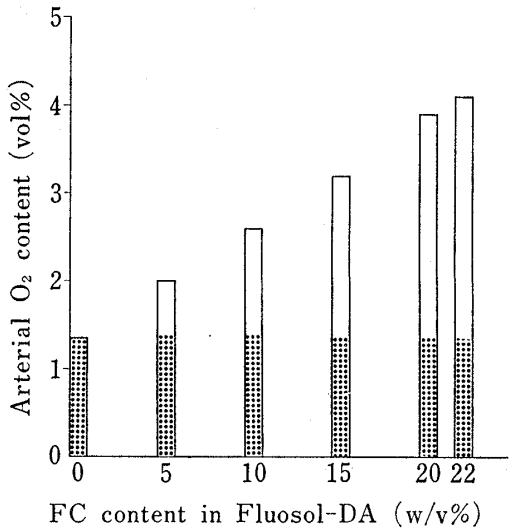


Fig. 8. Arterial O_2 Content at Hct 1 and Po_2 400 mmHg

Ten kinds of emulsions with different concentration of FCs, all made iso-oncotic with HES, were prepared with the medium of Krebs Ringer bicarbonate buffer solution, pH 7.4, as shown in Table VI. The highest concentrations were 28% in Fluosol-43 and 22% in Fluosol-DA.

Survival hours of rats were observed after the exchange transfusion repeated 35 times until the animals reached hematocrit 1%. The animals were kept in an oxygen chamber as mentioned above. Survival hours of rats receiving different kinds of emulsions are shown in Fig. 6 and 7. The animals usually became fully reactive and responsive, and were started on a regular diet 3 to 5 hr after termination of the repeated exchange transfusion. The longest survival hours of the animals resulted from the emulsions of 20% (w/v) concentration of the FCs of both of Fluosol-43 and -DA, which were remarkably effective compared with the non-FC control and surpassing the emulsions of higher concentrations.

To clarify the cause of this finding, oxygen content in arterial blood of rats after the exchange transfusion to make hematocrit 1% was measured. The arterial O_2 content was found almost proportional to the concentration of the FCs in the emulsions given as shown in Fig. 8 where the stippled bars represent O_2 content of rat's own blood at hematocrit 1% and the blank bars O_2 content carried by Fluosol-DA.

The apparent contradiction in the O_2 content of arterial blood and survival hours of rats indicates the necessity of further studies taking into consideration the increased viscosity of the emulsions and decreased O_2 transport capacity in circulation.

Discussion

Accumulation of FCs in various organs and their long-term effect had been the major problem in their use as a blood substitute in humans. A way of solving this problem was found by Clark and Naito[6] in 1972 by selecting FDC out of the heretofore known FCs on account of its short-term retention in tissues of organs after intravenous injection of its emulsion. Egg yolk phospholipid was selected as the emulsifying agent as Pluronic F68 known as the best agent for FC-43 cannot afford a stable emulsion of FDC. However, FDC emulsified with egg yolk phospholipid was later found to be unstable in circulating blood, as coat of FDC particles in the emulsion is rapidly lost, particles easily fused into larger mass, and the life-saving effect as the oxygen carrier was found to be much less than that of FC-43/Pluronic F68 emulsion as a consequence.[7]

To conquer the drawback of the FDC-phospholipid emulsion, Yokoyama invented a new blend of FDC and FBA (70: 30), both are fairly well eliminated from tissue of organs, and emulsified with Pluronic F68 affording an emulsion as stable as FC-43/Pluronic F68 emulsion *in vitro* and *vivo*.

Many investigators[2–4,6] have endeavored to find the use of FC compounds as the substituted of red cells utilizing the high oxygen solubility of the substance. The aimed application has been proved already in the preparation of isolated organs.[8]

To investigate the effect of the substance for prolongation of survival or life-saving, the stepwise exchange transfusion or hemodilution making hematocrit below the known limit (12% in human, 10% in dog[9]) affords the most proper estimation. Ohyanagi, *et al.*[10] have proved the effect of FC emulsions for life-saving of dogs hemodiluted to Hct=10% with this method.

7) R. Naito and K. Yokoyama, *Proc. Postcongr. Symp. X Int. Congr. Nutr.*, 55 (1975).
8) *a*) T. Suyama, K. Yokoyama, T. Matsumoto, Y. Odaka, M. Watanabe, H. Okamoto, R. Watanabe, and R. Naito, *Nippon Yuketsu Gakkai Zasshi*, **18**, 122 (1971); *b*) H. Brown and W.G.M. Hardison, *Surgery*, **71**, 388 (1974); *c*) M. Nakaya, *Jinko Zōki*, **4**, 283 (1975).
9) M. Takaori and P. Safar, *Arch. Surg.*, **92**, 743 (1966).
10) H. Ohyanagi, S. Yamashita, M. Sekita, S. Sugihara, K. Matsumoto, A. Ishii, S. Okumura, M. Shirakawa, and T. Mitsuno, *Kokyu to Junkan*, **22**, 380 (1974).

The present study has shown how the FC emulsion should be used in combination with oncotic agents to obtain a better effect. The results of the experiments indicate the importance of oncotic agents, no less effective than FC emulsion per se. As far as blood vloume is well maintained by oncotic agents as homologous plasma or 6% HES solution, FDC is sufficiently effective to prolong survival even if its concentration in blood is low as 5% (w/v).

A new composition of FC emulsion, FDC+FBA blend/Pluronic F68 emulsion containing HES and the constituents of Krebs-Ringer bicarbonate buffer solution, was studied for its effect on prolongation of survival. The optimum concentration of the FC substances in this formula was found to be 20% (w/v) instead of 22% based on survival period of exchange-transfused rats. The same applied to Fluosol-43, 20% instead of 28%.

As for the rate of replacement volume of Fluosol-DA to the lost blood volume at each step of exchange transfusion, 1:1 was found to be the best. The supplementary infusion of Fluosol-DA 24 hr after completion of the exchange transfusion had an effect of longer survival, and the second supplement 24 hr thereafter resulted in the same effect.

In consideration of the effect of oncotic agents and action of bicarbonate buffer, isooncosis, isotonicity, and pH, Fluosol-DA can be an approach to the red cell substitute, so far as the best. How long the FC substances are retained in tissues of organs is being studied and so far it seems to be quite short.