J. Pharm. Pharmacol. 1987, 39: 592–598
Received November 19, 1986

Effect of moderate haemodilution with Fluosol-DA or normal saline on indocyanine green and (+)-propranolol kinetics

ROBERT P. SHREWSBURY*, LEE M. LEWIS, SHARON R. OLIVER AND CATHERINE L. WOMBLE

School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514, USA

Indocyanine green (ICG) and (+)-propranolol kinetics were determined in the rat following moderate (50%) blood exchange with either Fluosol-DA or 0·9% NaCl (saline). Rats received an intravenous ICG dose (5 mg kg^{-1}) before an intravenous dose of (+)-propranolol (2·5 mg kg^{-1}) 0·5, 24, 48 or 72 h after haemodilution and were compared with non-exchanged controls. Haemodilution with Fluosol-DA reduced the ICG elimination rate constant during the first 24 h while a significant reduction was seen 48 h after normal saline exchange. ICG clearances tended to be less than the control value, and were significantly reduced only at 24 h after Fluosol-DA exchange due to a reduced V_{area}. (+)-Propranolol half-life was significantly increased 48 and 72 h after saline exchange; (+)-propranolol clearance was only significantly reduced 72 h after Fluosol-DA exchange. ICG clearance may be reflecting a hypovolaemic change which occurs after haemodilution, which would reduce the hepatic blood flow. However, (+)-propranolol clearance was not altered, suggesting that the hepatic blood flow is not changed. It is possible that ICG clearance is changed due to alterations in its extraction ratio instead of hepatic blood flow changes.

Perfluorochemical (PFC) emulsions are being investigated as potential blood substitutes because of their plasma expanding property and their ability to dissolve oxygen (Gould et al 1985). Numerous animal studies have demonstrated that PFC emulsions can sustain life (Hardy et al 1983; Yokoyama et al 1984). PFC emulsions have been used clinically in man for blood loss replacement, oxygen delivery to ischaemic tissues, and severe anaemia (Ohyanagi & Saitoh 1982; Mitsuno et al 1982; Tremper et al 1985).

PFC particles are captured by the reticuloendothelial system (RES) and distributed primarily to the liver and spleen, and secondarily to the kidney, bone marrow, and lungs (Mitsuno et al 1984). The time course of PFC uptake in the rat liver shows that maximal storage occurs 2 days after PFC administration (Lutz & Metzenauer 1980; Lutz et al 1982). The accumulation of PFC particles has been demonstrated in Kupffer cells, hepatocytes, mononuclear phagocytes, and 'foamy' macrophages (Lutz et al 1982; Mitsuno et al 1984; Lowe & Bollands 1985). Upon release from these organs, PFCs are carried by monocytes to the lungs where exhalation is the primary route of elimination. Urine and faecal excretion of PFCs is insignificant and they are not metabolized (Geyer 1982, 1983).

* Correspondence.

The presence of PFCs in the liver may alter normal hepatic function such as microsomal enzymatic activity, hepatic blood flow, and/or biliary excretion. Studies with sulphamethazine (Kemner et al 1984a), phenobarbitone (Lutz & Wagner 1984), phenytoin (Matsumoto et al 1983; Matsumoto-Kikuchi et al 1983), and morphine (Kemner et al 1984b) suggest that hepatic metabolism may be altered after PFC administration or exchange. A time-related effect may also play a role in the total influence of PFC emulsions on drug disposition. It was reported that the penicillin half-life was significantly longer in the rat 0·5 h after a 25 mL Fluosol-DA exchange than 48 h after the exchange (Hodges et al 1983, 1984).

Studies with indocyanine green (ICG) have suggested that hepatic blood flow is decreased after a PFC exchange (Lutz & Metzenauer 1980; Bizot & Rink 1985). ICG is completely eliminated without metabolism via biliary excretion, and it is suggested that the elimination of doses of approximately 5 mg kg^{-1} is limited by hepatic blood flow (Paumgartner 1975). Thus its clearance has been widely used to measure this flow rate (Caesar et al 1961; Yokota et al 1976; Iga et al 1980). However, ICG has an extraction ratio of 0·36 in the rat (Yokota et al 1976), and therefore its clearance should be influenced most by changes in the free drug fraction and hepatic intrinsic clearance, and not hepatic blood flow (Øie & Benet 1980). One paper has

suggested that ICG is not an appropriate compound to measure blood flow (Keiding & Andreasen 1979). (±)-Propranolol is another compound often used to measure hepatic blood flow, and has an extraction ratio in excess of 0·9 in the rat after intravenous administration of doses from 2·5 to 12·5 mg kg^{-1} (Suzuki et al 1980). (±)-Propranolol clearance should be dependent upon blood flow and independent of changes in free drug fraction and intrinsic clearance (Øie & Benet 1980). The (−)-isomer of propranolol has been shown to reduce hepatic blood flow approximately 30% because of its β-adrenoceptor blocking activity, while the (+)-isomer has no such effect (Branch et al 1973; Suzuki et al 1980).

The effect of partial haemodilution with Fluosol-DA (Fluosol) or saline on the hepatic blood flow of the rat was studied using both ICG and (+)-propranolol. Fluosol is a commercially prepared emulsion containing two PFCs, perfluorodecalin and perfluorotripropylamine, that has been used in both animals and man. The intravascular half-life of the two PFCs in Fluosol is approximately 10 to 13 h in the rat, and they are undetectable in the blood after 2 days (Lutz & Metzenauer 1980; Yokoyama et al 1982). The half-life of perfluorodecalin in the rat liver, spleen, lungs, kidney, and adipose tissue is 7 to 8 days, while the perfluorotripropylamine half-life in the tissues is greater than 60 days (Geyer 1982; Yokoyama et al 1984; Mitsuno et al 1984).

MATERIALS AND METHODS

Materials

Fluosol was donated by Alpha Therapeutics Corp. (Los Angeles, CA) and prepared daily as directed within 0·5 h of use. ICG (Sigma Chemical Co., St Louis, MO) was dissolved in HPLC grade water (5 mg mL^{-1}) 5 min before each intravenous administration. 0·9% NaCl (saline) could not be used as a diluent as salting out of the ICG would occur. Only one lot of ICG was used to avoid variation in the impurity content (Heintz et al 1986). (+)-Propranolol, donated by Ayerst Laboratories, Inc. (New York, NY), was dissolved in HPLC grade water (2 mg mL^{-1}). *N*-Cyclodesisopropylpropranolol (CDP), was obtained from Pierce Chemical Co. (Rockford, IL). HPLC grade solvents and buffers were obtained from commercial vendors and filtered before use. Male Sprague-Dawley albino rats, 276–433 g, obtained from Charles Rivers, Inc. (Wilmington, MA), had free access to food and water.

Methods

ICG and (+)-propranolol kinetics were examined in unexchanged rats and in rats moderately exchanged with either Fluosol or saline in parallel designed studies. Moderate blood exchange was used to avoid the need for supplemental oxygen. Saline exchanged groups were included to differentiate between changes in ICG and (+)-propranolol disposition due to the Fluosol itself and changes due to haemodilution alone.

A silastic cannula was implanted in the right jugular vein under light ether anaesthesia 24 h before any exchange or drug administration. Patency of the cannula was maintained daily with heparinized saline (20 units mL^{-1}). Rats in group I were not exchanged but were dosed 24 h after surgery (see Table 1). To demonstrate that cannulation alone did not alter ICG and (+)-propranolol disposition, two additional groups were not exchanged but received the compounds 72 and 96 h after surgery. Rats were partially exchanged with Fluosol or saline (40 mL kg^{-1}) and received the drugs 0·5, 24, 48 or 72 h after the exchange. The exchange procedure has previously been reported (Shrewsbury 1986).

Table 1. Treatment groups and average haematocrit at ICG dosing.

Group	Exchange fluid	Time between exchange and ICG dosing (h)	Mean (s.d.) haematocrit at time of ICG dosing (%)
I	None (control)		38·3 (7·0)
II	Fluosol-DA	0·5	22·8 (8·1)*
III	Fluosol-DA	24	24·4 (2·4)*
IV	Fluosol-DA	48	24·5 (8·2)*
V	Fluosol-DA	72	28·7 (3·7)*
VI	Saline	0·5	21·7 (6·3)*
VII	Saline	24	18·4 (2·5)*
VIII	Saline	48	20·4 (1·7)*
IX	Saline	72	28·2 (2·9)*

*$P \leq 0·05$.

Rats received 5 mg kg^{-1} i.v. ICG followed 30 min later with 2·5 mg kg^{-1} i.v. (+)-propranolol. After ICG dosing, blood samples (0·1 mL) were collected at 1, 1·5, 2, 3 and 4 min. The blood samples were placed in polystyrene cuvettes (10 × 10 × 45 mm) containing heparin (100 units mL^{-1} of blood). 1 mL of an aqueous mixture of 0·25% human serum albumin (Sigma Chemical Co., St, Louis, MO) and 0·20% sodium bicarbonate (EM Science, Cherry Hill, NJ) was immediately added to stabilize ICG (Gathje et al 1970). The mixture was briefly shaken, and the transmittance at 800 nm was determined by a visible spectrophotometry. Standard curves for ICG

were prepared daily using methanolic stock solutions and whole blood, 50 : 50 blood and Fluosol, or 50 : 50 blood and saline as appropriate. Standard curves were linear over the concentration range of 50–10 μg mL^{-1} with correlation coefficients of 0·93 or better.

(+)-Propranolol blood samples (0·1 mL) were collected at 10, 20, 30, 45, 60, 90 and 120 min in polypropylene tubes containing heparin (100 units mL^{-1} of blood) and frozen for subsequent analysis. The heparin concentration was less than that reported to cause artifactual changes in the percentage plasma protein binding of (±)-propranolol (Wood et al 1979). For HPLC analysis, the 100 μL blood sample was thawed, 20 μL of CDP (400 ng mL^{-1} in methanol, internal standard) and 100 μL 2 M NaOH was added, and extracted into 1 mL ethyl ether. The mixture was shaken for 15 min, centrifuged at approximately 12 000*g* for 2 min, and the organic layer aspirated with a Pasteur pipette. The organic solvent was evaporated under nitrogen, reconstituted in 100 μL methanol and injected.

(+)-Propranolol concentrations were determined by HPLC after the method of Gal et al (1985) using a 10 μm C_{18} (250 mm × 4·6 mm i.d.)Alltech column and a mobile phase of 900 mL water, 900 mL methanol, 300 mL acetonitrile, 400 μL phosphoric acid, and 2·3 g monobasic potassium phosphate (pH 3·9). The flow rate was 2·3 mL min^{-1} with fluorescence monitored (0·1 μA) at an excitation wavelength of 216 nm and an emission cut-off filter of 320 nm. The standard curves of peak area ratio were prepared using methanolic stock solutions and the appropriate blood mixture as described in the ICG procedure. Standard curves were linear over the range of 750 to 10 ng mL^{-1} with correlation coefficients of 0·94 or better.

Blood concentration-time ICG data were fitted with a non-linear regression calculator program yielding the intercept and slope of the line. From these values, all other one-compartment parameters were calculated as described in standard texts (Gibaldi & Perrier 1982). (+)-Propranolol data was fitted by SAAM27, a non-linear least-squares regression program (Berman & Weiss 1978) with blood concentrations weighted by the reciprocal of their squared value since the coefficient of variation was independent of the concentration assayed. 71% of the animals showed (+)-propranolol disposition fitted to a two compartment model; the remainder required a one compartment model to fit the data adequately. $t_{\frac{1}{2}}$ was calculated as $0{\cdot}693/K_{el}$ or $0{\cdot}693/\beta$ depending upon a one or two compartment model fitting, respectively. K_{el} is the slope of the line observed in the post distributive phase. V_{area} was calculated as dose/K_{el}(AUC) or dose/β (AUC) depending upon a one or two compartment fitting, where AUC is the sum of the pre-exponential coefficient divided by the exponent as required by the different models. The significance of difference between any group was assessed with the Wilcoxon Rank Sum Test (SAS Institute, Inc., Cary, NC) with a probability level of $P \leq 0{\cdot}05$ considered statistically significant.

RESULTS

There was no significant difference in any ICG or (+)-propranolol parameter in the three unexchanged (control) groups, and therefore the group dosed 24 h after cannulation was taken as the control for this report. All animals underwent the exchange procedure and the ICG and (+)-propranolol data collection protocol as outlined with a 100% survival rate. Animals generally lost weight as a result of either Fluosol or saline exchange; however, 17% of the animals in III to V and VII to IX showed a weight gain and 10% had no weight change. There was no immediate weight change in II and VI. The average weight loss was 1·8 g in III, 14·0 g in IV, 9·0 g in V, 8·2 g in VII and 3·6 g in VIII. Group IX had an average weight gain of 1·6 g.

The haematocrit before dosing with ICG was one-half of that before exchange, indicating a 50% blood exchange had been achieved (see Table 1). The haematocrit remained depressed at 72 h after exchange with either Fluosol or saline. Group II had an average fluorocrit (Geyer 1983) of 4·6%; fluorocrits were not observed in any other Fluosol-exchanged group.

ICG blood concentrations displayed a one-compartment disposition as expected. The mean ICG parameters are summarized in Table 2. Haemodilution with either fluid reduced the ICG K_{el} and increased the corresponding $t_{\frac{1}{2}}$ in all groups compared with control with significant changes 48 h after saline exchange and 0·5 and 24 h after Fluosol exchange. V_d tended to be greater than control in groups II, VI, VII and VIII, with a significant increase noted 48 h after Fluosol exchange (IV). ICG Cl values, either expressed as total body clearance or clearance per gram of liver weight, tended to be less than control, with only group III showing a significant reduction. Liver weight did increase with time after Fluosol exchange, while no such trend was seen with saline-exchanged animals.

The mean (+)-propranolol disposition parameters

Table 2. Averaged disposition parameters of indocyanine green.

	Groups								
Parameter	I	II	III	IV	V	VI	VII	VIII	IX
N	5	5	4	5	5	4	5	5	5
K_{el}	1.2†	0·4*	0·5*	0·7	1·0	1·0	0·9	0·5*	0·8
(min^{-1})	0.7	0·2	0·1	0·4	0·6	0·5	0·5	0·2	0·3
$t_{\frac{1}{2}}$	0·7	1·8*	1·4*	1·2	1·0	0·8	1·0	1·8*	1·0
(min)	0·3	0·5	0·3	0·7	0·5	0·3	0·6	1·0	0·6
V_d	22·8	42·5	17·2	44·8*	16·9	25·5	29·4	32·1	18·7
(mL kg^{-1})	10·2	29·8	6·7	16·5	7·1	13·5	21·2	19·7	6·8
Cl	24·5	15·7	8·5*	25·6	13·5	21·7	21·8	16·5	14·6
(mL min^{-1} kg^{-1})	10·6	9·2	1·6	6·5	5·1	7·1	15·4	15·2	7·1
Cl	0·6	0·4	0·2*	0·5	0·3	0·6	0·5	0·4	0·3
(mL min^{-1} g^{-1} liver)	0·3	0·2	0·1	0·1	0·1	0·2	0·4	0·4	0·1
Liver weight	14·2	14·6	16·1	16·8	16·8	14·1	14·6	14·0	13·6
(g)	1·7	3·0	1·5	2·2	2·1	1·4	0·9	2·2	2·3

† Mean with s.d. *$P \leq 0.05$.

Table 3. Averaged disposition parameters of (+)-propranolol.

	Groups								
Parameter	I	II	III	IV	V	VI	VII	VIII	IX
N	5	5	4	5	5	4	5	5	5
$t_{\frac{1}{2}}$	30·6†	34·7	21·9	48·2	32·8	22·1	21·3	56·7	52·7*
(min)	8·6	5·4	12·4	25·2	6·5	5·4	16·6	61·7	15·0
V_{area}	1014·0	1849·2	631·3	1955·2	491·4*	706·7	576·1*	2220·8	1551·4
(mL kg^{-1})	411·9	1317·1	443·7	1192·7	275·1	349·6	476·6	3334·2	908·0
Cl	23·2	27·2	19·6	26·1	10·4*	22·8	20·6	22·1	19·9
(mL min^{-1} kg^{-1})	6·7	15·1	4·3	8·1	5·5	11·2	10·0	15·2	8·3
Cl	0·6	0·7	0·4	0·5	0·2*	0·6	0·5	0·6	0·5
(mL min^{-1} g^{-1} liver)	0·2	0·4	0·1	0·2	0·1	0·3	0·2	0·4	0·2

† Mean with s.d. *$P \leq 0.05$.

are summarized in Table 3. Its $t_{\frac{1}{2}}$ was not significantly different from control after Fluosol exchange although it was reduced to its greatest extent 24 h after exchange (III). The $t_{\frac{1}{2}}$ was initially reduced at 0·5 and 24 h after saline exchange (VI, VII), but dramatically increased after 48 and 72 h. V_{area} after Fluosol exchange initially increased, then tended to decrease, while it was initially decreased and then increased after saline exchange. (+)-Propranolol Cl, whether expressed as total body clearance or clearance per gram of liver weight, was significantly different from control only at 72 h after Fluosol exchange.

DISCUSSION

Numerous investigators have demonstrated that animals can survive partial blood exchange with Fluosol without supplemental oxygen (Hodges et al 1983, 1984). A 71% Fluosol exchange without oxygen has been reported (Kemner et al 1984b). Rats in the present study underwent approximately a 50% exchange with Fluosol or saline without casualties. Although most animals lost weight as a result of the exchange, long term studies have shown that exchanged animals grow comparably with unexchanged animals (Watanabe et al 1979).

Haematocrits were reduced approximately 50% as a result of the exchange protocol and levels remained depressed through 72 h. Previous studies have shown that the haematocrit remains depressed longer after a one third exchange compared with a near total exchange (Zucali et al 1979; Gould et al 1983; Mitsuno et al 1984). It has been proposed that animals of the size used in this study have slower haematopoeisis than smaller animals (Geyer 1982).

ICG K_{el} was significantly decreased for 24 h after Fluosol exchange but at only 48 h after saline exchange. In a previous study, ICG K_{el} was determined at various times over 16 days after a one-third exchange with the Fluosol stem emulsion (Lutz &

Metzenauer 1980). K_{el} was decreased 6 h after exchange, reached a maximum reduction of 16% at 24 h, and returned to control values after 48 h. Fluosol or Fluosol-43 reduced ICG K_{el} 37% in the first hour after a 50% exchange (Bizot & Rink 1985). These studies taken together clearly show that haemodilution has a time-dependent effect on ICG elimination, creating the greatest influence within the first 24 h after exchange, and the effect dissipates with time.

ICG Cl, at the dose used in this study, has been reported to be limited by the hepatic blood flow (Paumgartner 1975). Cl was reduced in all groups except IV following haemodilution with either fluid, but only significantly reduced in III. The data imply that Fluosol decreases the hepatic blood flow for 24 h after exchange, consistent with the study of Lutz & Metzenauer (1980). It is well documented that haemodilution initially increases cardiac output due to a decreased viscosity of the circulatory fluid and decreased peripheral resistance (Kohno et al 1979; Faithfull et al 1984). After Fluosol exchange, there is increased blood flow to the brain, heart, liver, skeletal muscle, splanchic region, and kidneys (Handa et al 1983; Haneda et al 1983; Goslinga 1984). This is followed by a rapid decrease in the circulating blood volume which is reduced by one-third 3 h after the exchange and remains depressed for at least 24 h (Matsumoto et al 1977; Ohyanagi et al 1979; Watanabe et al 1979). Such a reduction in the circulating blood volume would subsequently decrease cardiac output. Thus it appears that ICG Cl may be reflecting these haemodynamic changes.

It could be argued that the ICG Cl is changing due to changes in the ICG extraction ratio and not alterations in the hepatic blood flow since PFC particles are sequestered in the hepatic RES system for several days (Lowe & Bollands 1985). The PFC particles present in the hepatocytes could alter the sinusoidal plasma membrane permeability, compete for the ICG carrier or binding site on the hepatocyte, or alter the association of ICG and the hepatocyte acceptor proteins. It has also been reported that Fluosol causes a decreased number of hepatocytes, as well as the hypertrophy of the remaining cells, within 1 to 4 days after exchange (Miller et al 1979; Lutz et al 1982). A decreased number of hepatocytes could decrease the ICG intrinsic clearance and thus its extraction ratio. Saline is not taken up in the RES (Mitsuno et al 1983), and ICG disposition is different in the saline-exchanged animals compared with Fluosol-exchanged animals.

(±)-Propranolol in a dose of 2·5 mg kg^{-1} has been shown to have an extraction ratio of 0·99 when intravenously administered to rats (Suzuki et al 1974, 1980). Therefore, (+)-propranolol Cl should be dependent solely on hepatic blood flow as the extraction ratio of both isomers is similar (Nies et al 1973). (+)-Propranolol Cl values were similar in all groups but V, which was significantly decreased by approximately 50%, suggesting that haemodilution with either fluid did not generally effect hepatic blood flow. There was no evidence of extrahepatic clearance of (+)-propranolol (Evans et al 1973) as Cl values were equal to or less than reported values of hepatic blood flow in the rat (Nies et al 1976; Pang & Gillette 1978; Suzuki et al 1980).

The significantly increased $t_{\frac{1}{2}}$ in group IX, and the similarly elevated value in VIII might suggest that (+)-propranolol elimination is reduced after saline exchange. The drug is extensively metabolized by the mixed function oxidases which require molecular oxygen (Routledge & Shand 1979; Walle et al 1984). After haemodilution, the oxygen capacity of the blood is necessarily reduced and oxygenation of tissues becomes dependent upon the oxygen capacity of the remaining blood plus the fluid used in haemodilution. Fluosol has been shown to have a higher oxygen capacity than plasma, dextrans, hydroxyethyl starch, lactated Ringer solution, Collin's solution, saline and water (Honda 1983; Kloner et al 1983; Peerless 1983; Tabuchi et al 1983; Gould et al 1985). Thus the oxygen delivery to the liver after saline exchange might be reduced compared with Fluosol-exchanged animals. ICG $t_{\frac{1}{2}}$ was likewise significantly increased in group VIII. However, ICG is completely eliminated via biliary excretion without biotransformation (Paumgartner 1975). Thus, a reduced oxygen capacity might decrease biliary excretion by changing the integrity of the specialized bile canaliculus membrane. Since it is not clear why an oxygen deficiency would only affect the membrane integrity 48 h after saline exchange, the response suggests that some other mechanism is responsible.

Haemodilution will decrease the blood protein content which will lead to an increased free drug fraction in the blood and an increased apparent volume of distribution (Gibaldi & Koup 1981). ICG V_d and (+)-propranolol V_{area} were increased in many groups, but not all. After Fluosol exchange, (+)-propranolol V_{area} showed the same pattern relevant to control as ICG V_d, but the pattern was not evident after saline exchange. Thus the decreased blood protein binding percentage did not produce the expected change in apparent volumes of

distribution. However, redistributions in the hepatic microcirculation have been reported to occur to prevent local anoxia (Kessler & Messmer 1975), or in response to low perfusion pressure and flow rates (Pang & Rowland 1977). Redistributions have also been reported to occur in cardiac tissues (Buckberg & Brazier 1975). Such redistributions may also partially explain the observed changes in ICG and (+)-propranolol $t_{\frac{1}{2}}$. The intact hepatocyte hypothesis suggests that an apparent reduction in the number of functioning hepatocytes can be accomplished when blood flow is shunted away from the liver (McLean et al 1978; Oie & Benet 1980). Hepatic redistribution could therefore contribute to a decreased ICG and (+)-propranolol elimination by shunting blood away from areas of high functioning hepatocyte density to low density areas, or by shunting blood away from the liver.

The present study has demonstrated that Fluosol exchange has an effect on ICG Cl for 24 h. The lack of change in (+)-propranolol Cl in the same groups suggests that the ICG Cl is being influenced more by blood protein binding changes or reductions in its extraction ratio than by hepatic blood flow. The extended PFC retention time in the liver could explain the ability of PFC to alter drug disposition for some time after a single exposure. A single mechanism of action is probably not responsible for all of the observed effects since changes in blood oxygen capacity, haemodynamics, and redistributions of organ blood flow patterns also occur after haemodilution.

Acknowledgement

Funded by a National Heart, Lung and Blood Institute Grant (HL33227).

REFERENCES

Berman, M., Weiss, M. F. (1978) SAAM Manual, U.S. DHEW Publication No. (NIH), 78–180, Washington, D.C.

Bizot, W. B., Rink, R. D. (1985) Experientia 41: 1127–1129

Branch, R. A., Nies, A. S., Shand, D. G. (1973) Drug Metab. Disp. 1: 687–690

Buckberg, G., Brazier, J. (1975) in: Messmer, K., Schmid-Schonbein, H. (eds) Intentional Hemodilution. Karger, Basel, pp 173–189

Caesar, S., Shaldon, S., Chiandussi, L., Guevara, L., Sherlock, S. (1961) Clin. Sci. 21: 43–47

Evans, G. H., Nies, A. S., Shand, D. G. (1973) J. Pharmacol. Exp. Ther. 186: 114–122

Faithfull, N. S., Fennema, M., VanAlphen, W. A., Smith, A. R., Erdmann, W., Essed, C. E., Kok, A., Lapin, R. (1984) Acta Anaesthesiol. Belgica 34 (Suppl.): 69–78

Gal, J., Rhodes, P. J., Nakata, L. M., Bloedow, D. C. (1985) Res. Comm. Chem. Path. Pharmacol. 48: 255–266

Gathje, J., Steuer, R. R., Nicholes, K. R. K. (1970) J. Appl. Physiol. 29: 181–185

Geyer, R. P. (1982) in: Frey, R., Beisbarth, H., Stosseck, H. (eds) Oxygen Carrying Colloidal Blood Substitutes: Fifth International Symposium on Perfluorochemical Blood Substitutes. W. Zuckschwerdt Verlag, Muchen, pp 214–219

Geyer, R. P. (1983) in: Bolin, R. B., Geyer, R. P., Nemo, G. J. (eds) Advances in Blood Substitutes Research: Progress in Clinical and Biological Research. Vol. 122. Alan R. Liss, New York, pp 157–168

Gibaldi, M., Koup, J. R. (1981) Eur. J. Clin. Pharmacol. 20: 299–305

Gibaldi, M., Perrier, D. (1982) Pharmacokinetics, 2nd edition. Marcel Dekker, New York, pp 17–20

Goslinga, H. (1984) Blood Viscosity and Shock: The Role of Hemodilution, Hemoconcentration and Defibrination. Springer-Verlag, New York, pp 34–35

Gould, S. A., Rosen, A. L., Sehgal, L. R., Moss, G. S. (1983) in: Bolin, R. B., Geyer, R. P., Nemo, G. J. (eds) Advances in Blood Substitutes Research: Progress in Clinical and Biological Research. Vol. 122, Alan R. Liss, New York, pp 331–342

Gould, S. A., Sehgal, L. R., Rosen, A. L., Sehgal, H. L., Moss, G. S. (1985) Ann. Emerg. Med. 14: 798–803

Handa, H., Nagasawa, S., Yonekawa, Y., Naruo, Y., Oda, Y. (1983) in: Bolin, R. B., Geyer, R. P., Nemo, G. J. (eds) Advances in Blood Substitutes Research: Progress in Clinical and Biological Research. Vol. 122, Alan R. Liss, New York, pp 299–306

Haneda, K., Honda, K., Kanno, M. (1983) in: Bolin, R. B., Geyer, R. P., Nemo, G. J. (eds) Advances in Blood Substitutes Research: Progress in Clinical and Biological Research. Vol. 122, Alan R. Liss, New York, p. 438

Hardy, R. N., Lowe, K. C., McNaughton, D. C. (1983) J. Physiol. 338: 451–461

Heintz, R., Svensson, C. K., Stoeckel, K., Powers, G. J., Lalka, D. C. (1986) J. Pharm. Sci. 75: 398–402

Hodges, G. R., Reed, J. S., Hignite, C. E., Snodgrass, W. R. (1983) in: Bolin, R. B., Geyer, R. P., Nemo, G. J. (eds) Advances in Blood Substitute Research: Progress in Clinical and Biological Research. Vol. 122, Alan R. Liss, New York, pp 430–431

Hodges, G. R., Worley, S. E., Kemner, J. M., Reed, J. S. (1984) Antimicrob. Agents Chemother. 26: 903–908

Honda, K. (1983) in: Bolin, R. B., Geyer, R. P., Nemo, G. J. (eds) Advances in Blood Substitutes Research: Progress in Clinical and Biological Research. Vol. 122, Alan R. Liss, New York, pp 327–330

Iga, T., Yokota, M., Sugiyama, Y., Awazu, S., Hanano, M. (1980) Biochem. Pharmacol. 29: 1291–1297

Keiding, S., Andreasen, P. B. (1979) Pharmacology 19: 105–110

Kemner, J. M., Snodgrass, W. R., Worley, S. E., Hodges, G. R., Melethil, S., Hignite, C. E., Tschanz, C. (1984a) Res. Comm. Chem. Path. Pharmacol. 46: 381–500

Kemner, J. M., Snodgrass, W. R., Worley, S. E., Hodges, G. R., Clark, G. M., Hignite, C. E. (1984b) J. Lab. Clin. Med. 104: 433–444

Kessler, M., Messmer, K. (1975) in: Messmer, K., Schmid-Schonbein, H. (eds) Intentional Hemodilution. Karger, Basel, pp 16–33

Kloner, R. A., Glogar, D., Rude, R. E., Khuri, S. F., Muller, J. E., Clark, L. C., Braunwald, E. (1983) in: Bolin, R. B., Geyer, R. P., Nemo, G. J. (eds) Advances in Blood Substitutes Research: Progress in Clinical and Biological Research. Vol. 122, Alan R. Liss, New York, pp 381–390

Kohno, S., Baba, T., Miyamuto, A., Niiya, K. (1979) in: Naito, R. (ed.) Proceedings of the Fourth International Sympoisum on Perfluorochemical Blood Substitutes. Excerpta Medica, Amsterdam, pp 361–371

Lowe, K. C., Bollands, A. D. (1985) Med. Lab. Sci. 42: 367–375

Lutz, J., Metzenauer, P. (1980) Pflugers Arch. 387: 175–181

Lutz, J., Metzenauer, P., Kunz, E., Heine, W. D. (1982) in: Frey, R., Beisbarth, H., Stosseck, K. (eds) Oxygen Carrying Colloidal Blood Substitutes: Fifth International Symposium on Perfluorochemical Blood Substitutes. W. Zuckschwerdt Verlag, Munchen, pp 73–81

Lutz, J., Wagner, M. (1984) Artif. Organs 8: 41–43

Matsumoto, J., Bianchine, J., Thompson, R., Sharp, C., Andresen, B., Ng, K., Gerber, N. (1983) Proc. West. Pharmacol. Soc. 26:403–407

Matsumoto, T., Watanabe, M., Hamano, T., Hanada, S., Suyama, T., Naito, R. (1977) Chem. Pharm. Bull. 25: 2163–2171

Matsumoto-Kikuchi, J., Bianchine, J. R., Gerber, N. (1983) Pharmacologist 25: 151

McLean, A. J., McNamara, P. J., DuSouich, P., Gibaldi, M., Lalka, D. (1978) Clin. Pharmacol. Ther. 24: 5–10

Miller, M. L., Moore, R. E., Clark, L. C. (1979) in: Naito, R. (ed.) Proceedings of the Fourth International Symposium on Perfluorochemical Blood Substitutes. Excerpta Medica, Amsterdam, pp 81–97

Mitsuno, T., Ohyanagi, H., Naito, R. (1982) Ann. Surg. 195: 60–69

Mitsuno, T., Tabuchi, Y., Ohyanagi, H., Sugiyama, T. (1983) in: Bolin, R. B., Geyer, R. P., Nemo, G. J. (eds) Advances in Blood Substitutes Research: Progress in Clinical and Biological Research. Vol. 122, Alan R. Liss, New York, pp 257–263

Mitsuno, T., Ohyanagi, H., Yokoyama, K. (1984) Artif. Organs 8: 25–33

Nies, A. S., Gwyn, E. H., Shand, D. G. (1973) J. Pharmacol. Exp. Ther. 184: 716–720

Nies, A. S., Shand, D. G., Wilkinson, G. R. (1976) Clin. Pharmacokinet. 1: 135–155

Ohyanagi, H., Saitoh, Y. (1982) in: Frey, R., Beisbarth, H., Stosseck, K. (ed) Oxygen Carrying Colloidal Blood Substitutes: Fifth International Symposium on Perfluorochemical Blood Substitutes. W. Zuckscherdt Verlag, Munchen, pp 178–186

Ohyanagi, H., Sekita, M., Yokoyama, K., Itoh, T., Toshima, K., Ohamoto, M., Kawa, Y., Mitsuno, T. (1979) in: Naito, R. (ed.) Proceedings of the Fourth International Symposium on Perfluorochemical Blood Substitutes. Excerpta Medica, Amsterdam, pp 373–389

Øie, S., Benet, L. Z. (1980) Ann. Report Med. Chem. 15: 277–287

Pang, K. S., Gillette, J. R. (1978) Drug Metab. Disp. 6: 567–576

Pang, K. S., Rowland, M. (1977) J. Pharmacokinet. Biopharm. 5: 655–680

Paumgartner, G. (1975) Schweiz. Med. Wschr. (Suppl. 17) 105: 5–30

Peerless, S. J. (1983) in: Bolin, R. B., Geyer, R. P., Nemo, G. J. (eds) Advances in Blood Substitutes Research: Progress in Clinical and Biological Research. Vol. 122, Alan R. Liss, New York, pp 353–362

Routledge, P. A., Shand, D. G. (1979) Clin. Pharmacokinet. 4: 73–90

Shrewsbury, R. P. (1986) J. Pharm. Pharmacol. 38: 647–652

Suzuki, T., Isozaki, S., Ishida, R., Saitoh, Y., Nakagawa, F. (1974) Chem. Pharm. Bull. 22: 1639–1645

Suzuki, T., Isozaki, S., Ohkuma, T., Rikihisa, T. (1980) J. Pharm. Dyn. 3: 603–611

Tabuchi, Y., Ohyanagi, H., Nakaya, S., Usami, M., Hattori, M., Suenaga, S., Saitoh, Y. (1983) in: Bolin, R. B., Geyer, R. P., Nemo, G. J. (eds) Advances in Blood Substitutes Research: Progress in Clinical and Biological Research. Vol. 122, Alan R. Liss, New York, pp 452–453

Tremper, K. K., Levin, E. M., Waxman, K. (1985) Int. Anesthesiol. Clin. 23: 185–197

Walle, T., Walle, U. K., Wilson, M. J., Fagan, T. C., Gaffney, T. E. (1984) Br. J. Clin. Pharmacol. 18:741–747

Watanabe, M., Hanada, S., Yano, K., Yokoyama, K., Suyama, T., Naito, R. (1979) in: Naito, R. (ed.) Proceedings of the Fourth International Symposium on Perfluorochemical Blood Substitutes. Excerpta Medica, Amsterdam, pp 347–358

Wood, M., Shand, D. G., Wood, A. J. J. (1979) Clin. Pharmacol. Ther. 25: 103–107

Yokota, M., Iga, T., Awaze, S., Hanano, M. (1976) J. Appl. Physiol. 41: 439–441

Yokoyama, K., Watanabe, M., Naito, R. (1982) in: Frey, R., Beisbarth, H., Stosseck, H. (eds) Oxygen Carrying Colloidal Blood Substitutes: Fifth International Symposium on Perfluorochemical Blood Substitutes. W. Zuckschwerdt Verlag, Munchen, pp 214–219

Yokoyama, K., Suyama, T., Okamoto, H., Watanabe, M., Ohyangi, H., Yoichi, S. (1984) Artif. Organs 8: 34–40

Zucali, J. R., Mirand, E. A., Gordan, A. S. (1979) J. Lab. Clin. Med. 94: 742–746