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Effects of a Perfluorochemical Blood Substitute on Diazepam Binding by Human Albumin

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Abstract — The binding of 0·4 μg mL^{-1} diazepam and 0·75 mol diazepam mol^{-1} of albumin by a perfluorochemical (PFC) emulsion, Fluosol-DA, 20%, by human serum albumin (HSA), and by their mixtures, has been examined at ambient temperature. The concentration of free diazepam was determined by standard centrifugation followed by supernatant ultrafiltration. Non-specific loss of diazepam occurred to the ultrafiltration device. This loss was independent of drug concentration and a correction factor was employed to calculate the true free diazepam concentration. Diazepam was extensively bound by the PFC emulsion. The percent free diazepam increased as the emulsion concentration decreased, while the binding of diazepam appeared to be independent of drug concentration. Diazepam did not partition into the pure PFC liquids, indicating that emulsion-bound diazepam is only associated with the emulsifiers of the droplets. Diazepam was extensively bound by HSA and the percent free diazepam increased as drug concentration increased or as HSA concentration decreased. The PFC emulsion significantly displaced HSA bound diazepam in all mixtures examined. Studies with the individual and combined components of the emulsion indicated that this displacement is largely attributed to the oleic acid component and, to a much smaller degree, the Pluronic F-68 component of the emulsion.

As a result of their ability to transport significant amounts of oxygen, perfluorochemical (PFC) emulsions have emerged for use as blood substitutes with potential use in a wide variety of disorders. These include myocardial and brain ischaemia, carbon monoxide poisoning, and sickle cell anaemia (Parsons 1985). One commercially available product (Fluosol-DA, 20%, Green Cross, Osaka, Japan) has been used clinically in several situations, including resuscitation, refusal of blood transfusion, preoperative anaemia, postpartum haemorrhage, carbon monoxide inhalation, and cerebral hypoxia (Editorial 1986; Faithfull 1987; Lowe 1987).

A PFC emulsion may alter the pharmacokinetics of other drugs. In moderately to highly exchanged transfused rats, PFC emulsions alter the pharmacokinetics of aspirin (Houck & Gerber 1984), salicylic acid (Houck & Gerber 1984), sulphamethazine (Kemner et al 1984a), morphine (Kemner et al 1984b), antipyrine (Shrewsbury et al 1986a), phenytoin (Matsumoto-Kikuchi et al 1983; Shrewsbury et al 1986b), indocyanine green (Shrewsbury et al 1987), and (+)-propranolol (Shrewsbury et al 1987). The pharmacokinetics of ampicillin (Shrewsbury 1986) and penicillin (Kemner et al 1984a) were unaltered in rats moderately exchanged transfused with Fluosol-DA.

In a study with four rats moderately exchanged transfused with Fluosol-DA, there was no significant difference in diazepam pharmacokinetics relative to control values (Kemner et al 1984a). However, plasma diazepam concentrations were lowered during the first several hours in Fluosol-DA-treated animals (Kemner et al 1984a). This could possibly reflect an increase in free fraction resulting in a shifting of drug to extravascular tissues and/or binding of drug by the PFC emulsion. However, factors other than plasma protein binding are the major determinants of diazepam pharmacokinetics (Greenblatt et al 1980; Sellers et al 1982).

One possible mechanism of altered drug pharmacokinetics in the presence of a PFC emulsion is altered plasma protein binding. The change in the volume of distribution of salicylic acid (Houck & Gerber 1984) and sulphamethazine (Kemner et al 1984a) in the presence of a PFC emulsion was possibly due to decreased plasma protein binding. The plasma protein binding of drugs could be altered in the presence of a PFC emulsion by several mechanisms. The low, but measurable, solubility of various substances in PFC liquids may be relevant to drug transport (Riess & LeBlanc 1982). Any drug bound by the emulsion droplets would be removed with the droplet from the vascular system by the reticuloendothelial system. In addition to changes in drug binding resulting from plasma protein dilution 1, PFC or emulsion components may displace plasma protein bound drug by direct and/or indirect mechanisms. Fluorochemicals used as aerosol propellants are bound by human albumin (Chiou & Hsia 1975). Emulsifiers used in PFC emulsions are surfactants, and this general class of compounds is strongly bound by proteins (Steinhardt & Reynolds 1969).

The effect of a PFC emulsion on the in-vitro binding of warfarin by human albumin has been studied. Warfarin is a model anionic drug which serves as a marker for one of the few high affinity drug binding sites on HSA (Sudlow et al 1975, 1976; Fehske et al 1981; Sjoholm 1986). Warfarin was weakly bound by the PFC emulsion (Parsons et al 1985) through an interaction with the emulsifiers of the droplets (Parsons 1987). Dilution of 4% HSA solutions to less than 50% v/v with PFC emulsion resulted in significant increases in percent free warfarin (Parsons et al 1985; Parsons 1987). This increase in percent free warfarin is attributed to the oleic acid component and, to a much smaller degree, the Pluronic

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F-68 component of the emulsion (Parsons & Nadkarni 1987).

The binding of prednisolone, a model non-ionic drug, was also examined in the presence of the PFC emulsion. Prednisolone was weakly bound by the PFC emulsion through an association with the emulsifiers of the droplets (Parsons 1986). Although this binding by the emulsion was weak, it was significant even in the presence of HSA. Emulsion binding of prednisolone partially offset the increase in percent free prednisolone which occurred upon, and was due only to, HSA dilution (Parsons 1986).

The present study further characterizes PFC emulsion binding of drug and drug binding by HSA in the presence of this PFC emulsion. Diazepam was chosen since it is extensively bound by HSA (Sellers et al 1982) and is an excellent marker for the second major high affinity drug binding site on HSA (Muller & Wollert 1979; Fehske et al 1981; Sjoholm 1986). Extensive studies of drug binding to specific sites on HSA have been conducted at room temperature (Sjoholm et al 1979; Sudlow et al 1975, 1976). Findings of these studies have correlated well with later studies conducted in-vivo (Kober et al 1978; Yoshikawa et al 1984).

Materials and Methods

All studies were conducted at ambient room temperature (24°C). All final solutions employed a 0·1M, pH 7·4 (±0·02) phosphate buffer. Two freshly prepared buffer systems were initially used. Both consisted of Na_2HPO_4, KH_2PO_4, and triple distilled water. Human albumin (HSA) solutions were prepared with essentially fatty acid-free HSA (Sigma) to which one mole of oleic acid, sodium salt (Sigma Chemical Co., St. Louis, MO, USA), mol^{-1} of HSA was added. Oleic acid was stored at −18°C in a sealed ampoule until immediately before use. Oleic acid was dissolved in triple distilled water and added directly to the HSA. A portion of 0·1M phosphate buffer, stem emulsion, or other component solution was added to complete the dissolution of HSA. A 0·2M phosphate buffer was then added in a volume equal to that of the aqueous oleic acid solution added. This method resulted in the rapid formation of clear solutions. Solutions without HSA only employed the 0·1M phosphate buffer.

Diazepam (Sigma Chemical Co., St. Louis, MO, USA) and [2-^{14}C] diazepam (Amersham, Arlington Heights, IL, USA) were used. Solutions of 0·4 $\mu g\ mL^{-1}$ diazepam consisted solely of [2-^{14}C] diazepam. Solutions of 0·75 mol diazepam mol^{-1} HSA were comprised of the required amount of diazepam and a trace amount of [2-^{14}C]diazepam. The latter concentration could be dissolved only in the presence of HSA and/or PFC stem emulsion. Diazepam was quantitated by liquid scintillation counting (LS-150, Beckman Instruments, Inc., Fullerton, CA, USA).

The PFC emulsion (Fluosol-DA, 20%, Green Cross, Osaka, Japan) was used as received. Since repetitive freezing and thawing of the emulsion should be avoided (Lutz 1983), it was thawed once, divided into 10 mL portions, and refrozen in glass vials. In this way, only the amount of PFC emulsion needed for a given experiment was subject to rethawing. The two annex solutions of electrolytes and hydroxyethylstarch supplied with Fluosol-DA were replaced with the phosphate buffer since the bicarbonate buffer of Fluosol-DA does not provide adequate pH control (Tomera & Geyer 1982). As used, the PFC emulsion contained 14·0% perfluorodecalin, 6·0% perfluorotripropylamine, 2·7% Pluronic F-68, 0·4% yolk phospholipids, 0·32% potassium oleate, and 0·8% glycerol, where all percentages are w/v. The average particle size of this product is 0·1-0·2 μm (Yang et al 1984).

Studies conducted with the emulsion components included glycerol (Fisher Scientific Co., Fair Lawn, NJ, USA), egg yolk phospholipids (Hepar Industries, Inc. Franklin, OH, USA), Pluronic F-68 (BASF Wyandotte Corp., Parsippany, NJ, USA), Pluronic F-68 plus sodium oleate, and all components combined. All component concentrations were equivalent to those in the emulsion, except for yolk phospholipids (1% of emulsion content), which have limited buffer solubility. Pluronic F-68 was included in the oleic acid solution for solubility reasons.

Recovery of 0·4 $\mu g\ mL^{-1}$ diazepam from the ultrafiltration tubes (Centrifree, Amicon Corp., Danvers, MA, USA) was examined. Ultrafiltrate was collected by centrifugation (Sorvall Superspeed, Sorvall, Inc., Norwalk, CT, USA) at 480g or 30g. Approximately 1 mL of sample was transferred to the ultrafiltration tubes, and from one to three rinse filtrates ranging from 100 to 775 μL were obtained. Three 25 μL samples of rinse filtrate were obtained for diazepam quantitation. The collection cup was wiped clean and the remaining sample in the tube was replaced with approximately 1 mL of fresh sample. The filtration process was repeated to collect three successive ultrafiltrates of approximately 100 μL each. Two 25 μL samples of each ultrafiltrate were obtained for diazepam quantitation. The collection cup was wiped clean after each filtration.

To determine if diazepam recovery from the ultrafiltration tube was dependent upon drug concentration, the percent recovery was determined at diazepam concentrations of 80, 60, 40, 30, 20, 10, 5, and 2% of 0·4 $\mu g\ mL^{-1}$ diazepam. The procedure was as described above, except a single rinse filtrate of approximately 225 μL was employed and centrifugation was at 480g.

Binding of 0·4 $\mu g\ mL^{-1}$ diazepam and 0·75 mol diazepam mol^{-1} HSA in the presence of various concentrations of the PFC stem emulsion, HSA, mixtures of the PFC emulsion with HSA, and HSA with various emulsion components was determined. These studies used standard centrifugation followed by supernatant ultrafiltration. A sample was placed in a glass centrifuge tube and five 25 μL samples were obtained for diazepam quantitation. The solution was centrifuged at 1475g to settle emulsion droplets. This is similar to the centrifugation of blood in the study of drug binding by erythrocytes. Directly subjecting the PFC emulsion to ultrafiltration results in partial blockage of the filter and prolonged filtration times (Parsons et al 1985). Centrifugation times varied from 20 to 30 min for the smallest to largest PFC emulsion concentrations examined, respectively. HSA does not undergo sedimentation at this centrifugal force. For solutions containing mixtures of PFC emulsion and HSA, three 25 μL samples of supernatant were obtained for diazepam quantitation. Approximately 1 mL of supernatant was transferred to the ultrafiltration tubes. A rinse filtrate of approximately 225 μL and three successive ultrafiltrates of approximately 100 μL each were obtained by

centrifugation at 480g as described above. Two 25 μL samples of each ultrafiltrate were collected for free diazepam quantitation. All binding studies were conducted in quadruplicate and comparisons were based on one way analysis of variance.

To study diazepam partitioning into the pure PFC liquids, all other constituents of the emulsion were removed by extraction (Parsons 1986). Partitioning of 0·4 μg mL^{-1} diazepam from 0·1M phosphate buffer into the pure PFC liquids was examined by mixing 0·5 mL of each for 30 min. Samples were centrifuged at 1475g for 30 min and five 25 μL samples of both the aqueous layer and PFC layer were obtained for diazepam quantitation. This experiment was repeated with sample agitation for 5 h. Both experiments were conducted in quadruplicate.

Results

There was no detectable loss of diazepam, HSA bound diazepam, or PFC emulsion bound diazepam to either the glass centrifuge tubes or the reservoir tube of the ultrafiltration device. A rinse filtrate of approximately 100 μL collected by centrifugation at 480g resulted in poor (approximately 80%) recovery of 0·4 μg mL^{-1} diazepam. The recovery in the three successive ultrafiltrates increased progressively from filtrate one to filtrate three. This indicated that non-specific loss of diazepam was occurring to the ultrafiltration device.

One to three rinse filtrates ranging from 100 to 775 μL were then obtained. After a rinse filtrate of approximately 200 μL, diazepam filtrate recovery was approximately 90%, and was constant for all three successive ultrafiltrates. For subsequent studies, a single rinse filtrate of approximately 225 μL followed by three successive ultrafiltrates of approximately 100 μL each was employed. The use of a lower centrifugal force (30g) did not further improve diazepam recovery, so 480g was used for all subsequent studies.

Studies with various concentrations of diazepam indicated that free drug recovery was independent of diazepam concentration. A plot of percent recovery versus diazepam concentration yielded a slope of 0·03, which did not statistically differ ($P > 0.05$) from zero. Thus, a correction factor may be employed to calculate the true free diazepam concentration. The mean percent recovery for all solutions was 89·9% ± 2·1. For all binding studies, the percent free diazepam was calculated as [(observed free diazepam concentration) (1/0·899)/(total diazepam concentration)](100).

For all binding studies, there was no significant difference in the free diazepam concentration in the three successive ultrafiltrates. This indicated that no further improvement in free drug recovery occurred after the 225 μL rinse filtrate. For studies with the PFC emulsion, this further indicated that emulsion droplets not settled by centrifugation did not detectably interfere with membrane passage of free drug. For studies with HSA, this demonstrated that the increase in HSA concentration that occurred during ultrafiltration did not detectably affect the free concentration of diazepam. The mean of the three successive ultrafiltrates was used to calculate free diazepam concentration.

The results for diazepam binding by the PFC emulsion are presented in Table 1. The diazepam concentrations of 32, 64, 98, and 128 μg mL^{-1} correspond to 0·75 mol diazepam mol^{-1} HSA for HSA concentrations of 25, 50, 75, and 100% v/v of 4% HSA, respectively. Diazepam was extensively bound by the PFC emulsion. The percent free diazepam decreased as the emulsion concentration increased. The percent free diazepam was independent of drug concentration ($P > 0.05$) at all emulsion concentrations except 50% v/v. When compared with the other data, the difference ($P < 0.005$) at the 50% v/v emulsion concentration appears coincidental. Overall, emulsion binding of diazepam appears to be independent of diazepam concentration.

Table 1. The Effect of PFC emulsion concentration on the free concentration of diazepam.

% v/v of PFC emulsion	Diazepam Concn (μg mL^{-1})	Mean (s.d.) percent free diazepam
25	0·4	33·4 (0·55)
25	128	34·7 (1·01)
50	0·4	22·1 (0·67)
50	128	23·7 (0·18)
75	0·4	17·2 (0·29)
75	128	18·0 (0·71)
100	0·4	15·4 (0·28)
100	32	16·2 (0·62)
100	64	15·3 (0·37)
100	96	15·0 (0·35)
100	128	15·7 0·44)

Diazepam did not significantly (< 5% of total) partition into the pure PFC liquids after either 0·5 or 5 h of agitation. Thus, emulsion bound diazepam is only associated with the emulsifiers of the droplets.

The results for HSA binding of diazepam are presented in Table 2. Diazepam was extensively bound by HSA. The percent free diazepam increases as drug concentration increases ($P < 0.005$), and as HSA concentration decreases ($P < 0.0005$). This has been demonstrated previously (Sellers et al 1982).

The results for diazepam binding by various mixtures of the PFC emulsion and 4% HSA are presented in Tables 3 and 4. The percent free diazepam was significantly increased ($P < 0.0005$) in all mixtures relative to HSA controls (Table 2). The largest percent increase in fraction free diazepam occurred at the lower drug concentration. Therefore, a PFC emulsion component(s) displaces albumin bound diazepam by a direct and/or indirect mechanism. Although the emulsion extensively binds diazepam, the emulsion only partially compensates for the increased free diazepam concentration that results from displacement of diazepam from HSA.

By sampling the supernatant after centrifugation, it is possible to approximate the percent of the total diazepam

Table 2. Effect of albumin concentration on the free concentration of diazepam.

% v/v of 4% HSA solution	Diazepam Concn	Mean (s.d.) percent free diazepam
100	0·4 μg mL^{-1}	0·21 (0·03)
100	0·75 mol mol^{-1} HSA	1·80 (0·06)
75	0·4 μg mL^{-1}	0·35 (0·04)
75	0·75 mol mol^{-1} HSA	3·07 (0·05)
50	0·4 μg mL^{-1}	0·47 (0·01)
50	0·75 mol mol^{-1} HSA	3·82 (0·08)
25	0·4 μg mL^{-1}	0·68 (0·02)
25	0·75 mol mol^{-1} HSA	5·94 (0·09)

Table 3. Diazepam (0·4 μg mL^{-1}) binding by mixtures of PFC emulsion with 4% human albumin solution.

% v/v of PFC emulsion	% v/v of 4% HSA solution	Mean (s.d.) % free diazepam	%[a] with PFC layer
25	75	0·49±0·03	0
50	50	1·52±0·06	0
75	25	11·3±0·20	46

[a]Approximate percent of total diazepam bound by the emulsion droplets.

Table 4. Diazepam (0·75 mol mol^{-1} HSA) binding by mixtures of PFC emulsion with 4% human albumin solution.

% v/v of PFC emulsion	% v/v of 4% HSA solution	Mean (s.d.) % free diazepam	%[a] with PFC layer
25	100	2·50±0·13	0
50	100	3·09±0·07	0
75	100	3·59±0·11	0
100	100	5·62±0·44	12
100	75	6·82±0·20	20
100	50	9·41±0·26	36
100	25	12·1±0·28	61

[a]Approximate percent of total diazepam bound by the emulsion droplets.

associated with the emulsion droplets and with HSA. The percent of total diazepam associated with the settled emulsion droplets will be slightly lower than the true percent bound by the emulsion since small emulsion droplets are not settled by the centrifugal force employed (Parsons 1986). In the mixtures examined, it appears that only when the PFC emulsion concentration is greater than 50% v/v and the % v/v ratio of PFC emulsion to 4% HSA is equal to, or greater than, one will diazepam be bound by the emulsion droplets. The concentration of diazepam associated with the emulsion droplets increases as this ratio increases. Thus, although the PFC emulsion extensively binds diazepam, the affinity of the emulsion for diazepam appears to be much less than the affinity of HSA for diazepam. However, in the solutions where a significant amount of diazepam is associated with the emulsion droplets, the actual displacement of albumin bound diazepam is more pronounced than it appears due to diazepam binding by the emulsion.

To determine which component(s) of the PFC emulsion significantly displaced HSA bound diazepam, the binding of 0·4 μg mL^{-1} diazepam and 0·75 mol diazepam mol^{-1} HSA by 1% HSA was determined in the presence of the various emulsion components. The results are presented in Table 5. Pluronic F-68 alone has a small, yet statistically significant ($P < 0·0005$), displacement effect. Pluronic F-68 combined with oleic acid dramatically displaces diazepam ($P < 0·0005$), with the mean percent free diazepam being 39·1% (1·38) and 45·9% (1·02) for 0·4 μg mL^{-1} diazepam and 0·75 mol diazepam mol^{-1} HSA, respectively. These figures are essentially identical ($P > 0·25$) to the values obtained with all combined components. Changes in HSA binding of diazepam in the presence of this PFC emulsion may thus be largely attributed to the oleic acid and, to a much smaller degree, Pluronic F-68 components of the emulsion.

Table 5. Effect of PFC emulsion components on HSA binding of diazepam.

Component[a]	Mean (s.d.) % free diazepam 0·4 μg mL^{-1} Diazepam	0·75 mol diazepam mol^{-1} HSA
1·0% HSA control	0·68 (0·02)	5·94 (0·09)
Glycerol	0·63 (0·02)	6·04 (0·15)
Phospholipid	0·75 (0·05)	6·13 (0·04)
Pluronic F-68	0·84 (0·03)[b]	7·80 (0·18)[b]
Pluronic F-68 + oleic acid	39·1 (1·38)[b]	45·9 (1·02)[b]
Combined components	39·5 (1·61)[b]	46·1 (0·86)[b]

[a]All results are with 1·0% HSA and component concentrations of 100% (1% for phospholipids) of the emulsion content.
[b]Significantly different from 1·0% HSA control ($P < 0·0005$).

Discussion

Drug binding by the PFC emulsion itself is currently difficult to predict. Diazepam was extensively bound by this emulsion, whereas prednisolone (Parsons 1986) and warfarin (Parsons 1987) were only weakly bound. Based on these limited results, it appears that weakly basic drugs may be more strongly bound by the PFC emulsion than neutral or acidic drugs. None of these three drugs partitioned from pH 7·4 buffer into the pure PFC liquids. Thus, bound drug was only associated with the emulsifiers of the droplets.

Although the PFC emulsion extensively binds diazepam, the affinity of the emulsion for diazepam appears to be much less than the affinity of HSA for diazepam. Relative to HSA controls, the percent free diazepam was significantly increased in all mixtures of HSA and emulsion examined. This increase was due to displacement of HSA bound diazepam by PFC emulsion components.

Studies with the individual components of the PFC emulsion demonstrated that displacement of HSA bound diazepam is largely attributed to the oleic acid and, to a much smaller degree, Pluronic F-68 components of the emulsion. The increases in percent free diazepam due to these individual components are much greater than those obtained with the intact emulsion. These results are likely due not only to the lack of diazepam binding by the components compared with the emulsion, but also to the fact that only a small amount of the oleic acid and Pluronic F-68 in the intact emulsion may be free to interact with HSA.

The present results with diazepam are in good agreement with those obtained previously with warfarin. Warfarin displacement from HSA was largely due to oleic acid and, to a small degree, to Pluronic F-68 (Parsons & Nadkarni 1987). The ability of oleic acid to displace albumin bound diazepam, primarily through an allosteric mechanism, has been well established (Naranjo & Sellers 1986; Sellers et al 1982; Wong & Sellers 1979). As was seen in the present study, fatty acids will induce a greater percent increase in fraction free diazepam at lower drug concentrations (Tsutsumi et al 1975). Oleic acid concentrations of less than one mol mol^{-1} HSA will displace albumin bound diazepam (Wong & Sellers 1979). For the studies with the emulsion components, the oleic acid concentration is approximately 7·7 mol mol^{-1} HSA. It is reasonable that this amount of oleic acid could induce the dramatic displacement of diazepam observed.

Other drugs which bind to the diazepam site on HSA could

potentially be displaced by this PFC emulsion. Fatty acids have been shown to significantly displace other drugs which bind to this high affinity site on albumin (Birkett et al 1977). Free serum levels of tryptophan, which binds with high affinity to this site on albumin (Fehske et al 1981), was significantly increased by the in-vitro addition of a lipidic emulsion (Intralipid) which increased free fatty acid levels (Pena et al 1983).

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References

Birkett, D. J., Myers, S. P., Sudlow, G. (1977) The fatty acid content and drug binding characteristics of commercial albumin preparations. Clin. Chim. Acta 85: 253–258

Chiou, W. L., Hsia, J. H. (1975) Fluorocarbon aerosol propellants V. Binding interaction with human albumin. J. Pharm. Sci. 64: 1052–1054

Editorial (1986) Blood substitutes: has the right solution been found. Lancet 1: 717-718

Faithfull, N. S. (1987) Fluorocarbons. Current status and future applications. Anaesthesia 42: 234–242

Fehske, K. J., Muller, W. E., Wollert, U. (1981) The location of drug binding sites in human serum albumin. Biochem. Pharmacol. 30: 687–692

Greenblatt, D. J., Allen, M. D., Harmatz, B. A., Shader, R. I. (1980) Diazepam disposition determinants. Clin. Pharmacol. Ther. 27: 301–312

Houck, J. A., Gerber, N. (1984) The influence of perfluorochemical artificial blood substitutes on the disposition of aspirin and salicylic acid in the rat. The Pharmacologist 26: 167

Kemner, J. M., Snodgrass, W. R., Worley, S. E., Hodges, G. R., Melethil, S., Hignite, C. E., Tschanz, C. (1984a) Effect of oxygen-carrying resuscitation fluids on the pharmacokinetics of antipyrine, diazepam, penicillin, and sulfamethazine in rats. Res. Comm. Chem. Path. Pharmacol. 46: 381–400

Kemner, J. M., Snodgrass, W. R., Worley, S. E., Hodges, G. R., Clark, G. M., Hignite, C. E. (1984b) Interaction of oxygen-carrying resuscitation fluids with morphine. J. Lab. Clin. Med. 104: 433–444

Kober, A., Jenner, A., Sjoholm, I. (1978) Differentiated effects of liver cirrhosis on the albumin binding sites for diazepam, salicylic acid, and warfarin. Biochem. Pharmac. 27: 2729- 2735

Lowe, K. C. (1987) Perfluorocarbons as oxygen-transport fluids. Comp. Biochem. Physiol. 87A: 825–838

Lutz, J. (1983) Studies on RES function in rats and mice after different doses of Fluosol. Prog. Clin. Biol. Res. 122: 197–208

Matsumoto-Kikuchi, J., Bianchine, J. R., Gerber, N. (1983) Pharmacokinetics of phenytoin in the rats treated with Fluosol-43. The Pharmacologist 25: 151

Muller, W. E., Wollert, U. (1979) Human serum albumin as a 'silent receptor' for drugs and endogenous substances. Pharmacology 19: 59–67

Naranjo, C. A., Sellers, E. M. (1986) Fatty acid modulation of drug binding to plasma proteins. in: Reidenberg, M., Erill, S. (eds) Drug-Protein Binding, Praeger, New York, pp 233–253

Parsons, D. L. (1985) Perfluorochemical blood substitutes. U.S. Pharm. Hosp. Ed. 10: H14-H21

Parsons, D. L. (1986) Effect of a perfluorochemical emulsion on prednisolone binding by human albumin. Biochem. Pharmacol. 35: 1395–1397

Parsons, D. L. (1987) Perfluorochemical emulsion effect on warfarin binding in fraction V human albumin. Arch. Int. Pharmacodyn. 286: 23–30

Parsons, D. L., Nadkarni, S. R. (1987) Effects of perfluorochemical emulsion components on human albumin binding of warfarin. Ibid 288: 165–174

Parsons, D. L., Ravis, W. R., Clark, C. R. (1985) Binding of warfarin by human albumin in the presence of a perfluorochemical blood substitute. Ibid 277: 4–14

Pena, J. M., Aulesa, C., Vinas, O., Bosch, J., Farriol, M., Schwartz, S. (1983) Intralipid and free plasma tryptophan in vitro. Rev. Esp. Fisiol. 39: 39–44

Riess, J. G., LeBlanc, M. (1982) Solubility and transport phenomena in perfluorochemicals relevant to blood substitutions and other biomedical applications. Pure Appl. Chem. 54: 2383–2406

Sellers, E. M., Naranjo, C. A., Khouw, V., Greenblatt, D. J. (1982) Binding of benzodiazepines to plasma proteins in: Usdin, E. Skolnick, P., Tallman, J., Greenblatt, D., Paul, S. (eds) Pharmacology of Benzodiazepines. Unwin Brothers/Gresham Press, Surrey, Great Britain, pp 271–284

Shrewsbury, R. P. (1986) Effect of moderate hemodilution with Fluosol-DA or normal saline on ampicillin kinetics in the rat. J. Pharm. Pharmacol. 38: 647–652

Shrewsbury, R. P., White, S. G., Pollack, G. M., Wargin, W. A. (1986a) Antipyrine kinetics following partial blood exchange with Fluosol-DA in the rat. Ibid 38: 883–887

Shrewsbury, R. P., Lewis, L. M., Oliver, S. R. (1986b) Phenytoin kinetics following partial Fluosol-DA or normal saline blood exchange. Pharm. Res. 3: 161S

Shrewsbury, R. P., Lewis, L. M., Oliver, S. R., Womble, C. L. (1987) Indocyanine green and d-propranolol kinetics following moderate Fluosol-DA or normal saline hemodilution. Ibid 4: 763

Sjoholm, I., Ekman, B., Kober, A., Ljungstedt-Pahlman, B., Sjodin, T. (1979) Binding of drugs to human serum albumin. Molec Pharmacol. 16: 767–777

Sjoholm, I. (1986) The specificity of drug binding sites on human serum albumin. in: Reidenberg, M. M., Erill, S. (eds) Drug-Protein Binding, Praeger, New York, pp 36–45

Steinhardt, J., Reynolds, J. A. (1969) in: Multiple Equilibria in Proteins, Academic Press, New York, pp 237–275

Sudlow, G., Birkett, D. J., Wade, D. N. (1975) The characterization of two specific drug binding sites on human serum albumin. Ibid 11: 824–832

Sudlow, G. Birkett, D. J., Wade, D. N. (1976) Further characterization of specific drug binding sites on human serum albumin. Mol. Pharmacol. 12: 1052–1061

Tomera, J. F., Geyer, R. P. (1982) Perfluorochemical perfusion of the rat heart: in vivo and in vitro. J. Mol. Cell. Cardio. 14: 573–585

Tsutsumi, E., Inaba, T., Mahon, W. A. (1975) The displacing effect of a fatty acid on the binding of diazepam to human serum albumin. Biochem. Pharmacol. 24: 1361–1362

Wong, G. B., Sellers, E. M. (1979) Intravascular factors affecting diazepam binding to human serum albumin. Ibid 28: 3265–3270

Yang, F. S., Caldwell, K. D., Giddings, J. C., Astle, L. (1984) Stability of perfluorocarbon blood substitutes determined by; sedimentation field-flow fractionation. Analyt. Biochem. 138: 488–494

Yoshikawa, T., Sugiyama, Y., Sawada, T., Iga, M., Hanano, S., Kawasaki, M., Yanagida, M. (1984) Effect of late pregnancy on salicylate, diazepam, warfarin, and propranolol binding: use of fluorescent probes. Clin. Pharm. Therap. 36: 201–208