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The Effect of Varying Percentages of Haemodilution with Fluosol-DA or Normal Saline on Antipyrine Metabolism in the Rat

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Abstract—Antipyrine disposition and metabolism in conscious, unrestrained rats after 25 or 50% haemodilution with Fluosol or normal (0·9% NaCl) saline is reported. Rats received an intravenous antipyrine dose (20 mg kg^{-1}) 0·5, 24, 48, or 72 h after haemodilution and its pharmacokinetic parameters have been compared with non-exchanged control animals. Haemodilution 25% with Fluosol initially depressed antipyrine metabolism for 24 h by decreasing the antipyrine urinary excretion rate constant and the formation rate constants of 4-hydroxyantipyrine (4-OH) and 3-hydroxymethylantipyrine (3-OHME). Metabolism was then increased for 48 and 72 h with a slight increase in all rate constants. Haemodilution 50% with Fluosol produced a similar pattern but with significant increases in the 3-OHME formation rate constant found at 48 and 72 h. Haemodilution 25% with saline reduced 4-OH formation for 48 h. Haemodilution 50% with saline significantly reduced antipyrine urinary excretion at all times. After a significant increase in the 4-OH and 3-OHME formation rate constants at 24 h following 50% haemodilution with saline, the rate constants were significantly decreased at 48 and 72 h. Haemodilution 25% with Fluosol significantly reduced the antipyrine V_d at 0·5 and 72 h. After haemodilution 50% with Fluosol, the V_d alternated between values greater and less than control throughout the 72 h. Haemodilution 25 or 50% with saline had little influence on V_d.

Perfluorochemical (PFC) emulsions are currently being investigated as potential blood substitutes or acellular oxygen-carrying substances because of their plasma expanding property and their ability to dissolve oxygen. Numerous animal studies have demonstrated that PFC emulsions can sustain life (Yokoyama et al 1984; Shrewsbury 1987). PFC emulsions have been used clinically in man for blood loss replacement, oxygen delivery to ischaemic tissues, and severe anaemia (Mitsuno et al 1982; Tremper et al 1982).

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). Upon release from these organs, monocytes carry the PFCs to the lungs where exhalation is the primary route of elimination. Urinary and faecal excretion of PFCs are insignificant and there is no metabolic degradation of these compounds (Geyer 1982).

The time course of PFC uptake in the liver shows that the maximal storage occurs two days after PFC administration (Lutz & Metzenauer 1980). The accumulation of PFC particles has been demonstrated in Kupffer cells, hepatocytes, mononuclear phagocytes, and "foamy" macrophages (Lutz et al 1982; Lowe & Bollands 1985). The presence of PFCs in the liver may alter normal hepatic function such as biliary excretion, hepatic blood flow, or microsomal and non-microsomal metabolism. Studies with indocyanine green have suggested that hepatic blood flow is compromised (Bizot & Rink 1985), although a recent (+)-propranolol study indicated that hepatic blood flow was not altered after 50% Fluosol-DA (Fluosol) haemodilution (Shrewsbury et al 1987b). The latter report suggested that the PFC particles may change the intrinsic capacity of the liver to extract and excrete indocyanine green. Studies with sulphamethazine (Kemner et al 1984b), pentobarbitone (Lutz & Wagner 1984), phenytoin (Matsumoto et al 1983; Shrewsbury et al 1987a), and morphine (Kemner et al 1984a) suggest that microsomal metabolism may also be compromised. The recent phenytoin study (Shrewsbury et al 1987a) also showed that the non-microsomal glucuronidation pathway was induced by partial Fluosol haemodilution.

Recent studies have indicated that the effect of partial Fluosol haemodilution is time-dependent (Shrewsbury 1987). For example, it was reported that antipyrine showed an altered pharmacokinetic pattern for 72 h consistent with enhanced hepatic microsomal enzymatic activity after partial Fluosol haemodilution (Shrewsbury et al 1986). Indocyanine green, (+)-propranolol, and phenytoin have also showed time-dependent changes in pharmacokinetic parameters after partial Fluosol or normal (0·9% NaCl) saline haemodilution.

The current investigation was undertaken to determine whether antipyrine pharmacokinetics are altered by different percentages of haemodilution with Fluosol or saline. Antipyrine is extensively metabolized by the microsomal mixed function oxidase system and is commonly used to assess hepatic metabolism in man and animals (Wilson et al 1982). It is distributed in total body water (Stevenson 1977), and its clearance is insensitive to changes in hepatic blood flow (Branch et al 1974). The urinary excretion of antipyrine and its major metabolites, 3-methylantipyrine (3-ME), 4-hydroxyantipyrine (4-OH), and 3-hydroxymethylantipyrine (3-OHME) were simultaneously determined to provide

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insight to alterations in the hepatic cytochrome P450 or cytochrome P448 activity after haemodilution.

Materials and Methods

Materials

Fluosol was donated by Alpha Therapeutic Corporation (Los Angeles, California), and was prepared as directed within 0·5 h of use. Antipyrine, phenacetin, 3-ME, 4-OH, and HPLC grade solvents and buffers were obtained from commercial sources and used without further purification. 3-OHME was the gift of Dr D. D. Breimer, University of Leiden. Male Sprague-Dawley rats, 250–450 g, were used; they had free access to food and water.

Methods

Antipyrine kinetics were examined in unexchanged rats and rats in which the blood had been diluted 25 or 50% with Fluosol or saline. The study protocol and procedures have been previously reported (Shrewsbury et al 1986). A 25% haemodilution was accomplished using 20 mL kg^{-1} of the haemodiluent, and the 50% haemodilution required 40 mL kg^{-1}. Antipyrine kinetics were examined at 0·5, 24, 48 and 72 h following haemodilution by administering a single intravenous dose (20 mg kg^{-1}) through the implanted cannula. (Fluosol haemodiluted groups are coded 0·5HF, 48HF and 72HF; saline groups are coded 0·5HS, 24HS, 48HS and 72HS. The unexchanged control group is coded CONT.) Urine was collected over sodium metabisulphite for 24 h.

Plasma antipyrine concentrations were determined by high pressure liquid chromatography as previously described (Shrewsbury et al 1986). In contrast to that original published procedure, the standard curves in this study were prepared using plasma from whole blood or plasma from animals which had been haemodiluted with the appropriate haemodiluent and percentage. The plasma concentration-time data were fitted to a one-compartment open model using SAAM27 (Berman & Weiss 1978) with the plasma concentrations weighted by the reciprocal of their squared value as the coefficient of variation was independent of the concentration assayed.

Urinary concentrations of antipyrine and its metabolites were determined by high pressure liquid chromatography using modifications of two published procedures (Danhof et al 1979a; Griffeth et al 1984). 1·0 mL of 0·5 M acetate buffer, pH 4·5, 40 mg of sodium metabisulphite, and 10 mg of β-glucuronidase (limpet acetone powder) were added to 0·5 mL urine. The samples were vortexed and incubated for 3 h at 37°C. 50 μL of internal standard (phenacetin 100 μg mL^{-1}) was added to each sample after incubation, and then divided in two 0·8 mL aliquots for extraction by acidic and basic procedures. For the acidic extraction (yielding 3-ME and 4-OH), 5 mL of methylene chloride–n-pentane (30:70 v/v) were added and the sample was briefly vortexed and centrifuged (2400 rev min^{-1}) for 5 min. The aqueous layer was frozen in dry ice and the organic layer collected and dried under nitrogen at 40°C. For the basic extraction (yielding 3-OHME and antipyrine) 200 mg of NaCl and 500 μL of 4 M NaOH were added. Methylene chloride (10 mL) was added and the sample was briefly vortexed and centrifuged (2400 rev min^{-1}) for 5 min. The aqueous layer was aspirated, the organic layer was then collected by transfer pipette and dried under nitrogen at 40°C. Both acidic and basic residues were reconstituted with 250 μL of methanol. Chromatographic separation was achieved with a 5 μm CN (250 mm × 4·6 mm i.d.) Zorbax column and a mobile phase of 97·5% water, 2·33% acetonitrile and 0·17% triethylamine (pH 4·0) at a flow rate of 1·4 mL min^{-1}. Slight adjustments in the mobile phase composition and flow rate were necessary from day to day due to column sensitivity. Ultraviolet absorbency was monitored at 254 nm and the peak area ratios were determined. Standard curves were prepared using urine from unexchanged animals or animals haemodiluted with the appropriate haemodiluent and percentage.

The amount of 3-ME, 4-OH, 3-OHME, and antipyrine excreted was determined as a percentage of the dose administered. The antipyrine urinary excretion rate constant (k_u) was calculated as:

$$k_u = (\%)_u * K_{el}$$

where $(\%)_u$ is the percent of the dose excreted as unchanged antipyrine in 24 h, and K_{el} is the overall elimination rate constant determined from the slope of the plasma concentration-time curve. The formation rate constants (k_f) of 3-ME, 4-OH, and 3-OHME were determined as:

$$k_f = (\%)_m * K_{el}$$

where $(\%)_m$ is the percent of the dose excreted as the metabolite. Metabolic clearance (Cl_m) of 3-ME, 4-OH, and 3-OHME was determined as:

$$Cl_m = (\%)_m * Cl$$

where Cl is the antipyrine clearance obtained from the plasma concentration-time data. Renal clearance (Cl_R) of antipyrine was calculated as:

$$Cl_R = (\%)_u * Cl$$

The Wilcoxon rank sum test (SAS Institute, Inc., Cary, NC) was used to test the significance level of each parameter of the haemodiluted group to the corresponding control. A probability level of $P \leq 0{\cdot}05$ was considered significant.

Results

All animals underwent the haemodilution procedure and data collection protocol without difficulty. Most animals lost weight as the result of haemodilution. After 25% haemodilution, average weight losses were : 24HF = 10·6 g; 48HF = 1·0 g; 72HF = 6·0 g; and 24HS = 8·6 g. Average weight gains were 48HS = 4·3 g and 72HS = 10·8 g. After 50% haemodilution, average weight losses were: 24HF = 3·2 g; 48HF = 13·2 g; 72HF = 14·6 g; 24HS = 8·0 g; 48HS = 8·4 g; and 72HS = 11·2 g.

Haematocrits were reduced approximately 25 or 50% as a result of the haemodilution protocol. After 25% haemodilution, the averaged group haematocrit was decreased 12 to 40% of the prehaemodilution value; after 50% haemodilution, the decrease was 45 to 56%. The haematocrits remained depressed through the 72 h experimental period.

Table 1. Averaged disposition parameters of antipyrine following 25% haemodilution with Fluosol (HF) or saline (HS).

	Treatment groups								
Parameters	CONT	0·5HF	24HF	48HF	72HF	0·5HS	24HS	48HS	72HS
N	5	5	5	5	4	5	5	4	5
K_{el}	0·0079†	0·0058*	0·0042*	0·0094	0·0120*	0·0066*	0·0056*	0·0052*	0·0079
(min^{-1})	0·0010	0·0004	0·0017	0·0045	0·0016	0·0005	0·0005	0·0012	0·0024
Half-life ($t_{\frac{1}{2}}$)	89·1	120·2*	208·2*	83·8	59·9*	106·3*	124·7*	141·5*	95·2
(min)	10·0	7·7	145·9	28·5	8·4	8·3	11·2	42·2	29·6
Volume (V_d)	823·3	568·7*	1013·5	710·5	526·3*	843·6	733·8	912·3	812·1
(mL kg^{-1})	97·1	93·2	243·6	207·3	117·6	95·6	122·7	180·1	345·0
Clearance (Cl)	6·4	3·3*	4·0*	6·1	6·3	5·5	4·1*	4·6*	6·1
(mL min^{-1} kg^{-1})	0·7	0·4	1·4	1·4	2·1	0·8	0·9	1·0	2·3

† Mean with s.d.
* Significantly different from control ($P \leq 0{\cdot}05$).

Table 2. Averaged disposition parameters of antipyrine following 50% haemodilution with Fluosol (HF) or saline (HS).

	Treatment groups								
Parameters	CONT	0·5HF	24HF	48HF	72HF	0·5HS	24HS	48HS	72HS
N	5	5	4	5	5	5	5	5	4
K_{el}	0·0079†	0·0052*	0·0110	0·0110*	0·0100	0·0072	0·0100*	0·0045*	0·0040*
(min^{-1})	0·0010	0·0015	0·0038	0·0018	0·0032	0·0020	0·0014	0·0004	0·0007
Half-life ($t_{\frac{1}{2}}$)	89·1	142·6*	67·8	63·3*	74·1	102·5	70·7*	154·9*	177·9*
(min)	10·0	42·9	23·6	9·5	26·5	31·9	10·1	12·4	39·8
Volume (V_d)	823·3	1223·9*	415·3*	1145·8*	482·6*	1042·0	1393·3*	966·8	921·8
(mL kg^{-1})	97·1	323·2	175·1	367·2	183·1	344·9	448·8	305·8	220·0
Clearance (Cl)	6·4	6·4	4·5	12·6*	4·7	7·1	13·4*	4·4	3·8*
(mL min^{-1} kg^{-1})	0·7	2·7	2·1	3·4	1·6	1·6	2·5	1·6	1·3

† Mean with s.d.
* Significantly different from control ($P \leq 0{\cdot}05$).

Table 3. Averaged disposition of urinary antipyrine and metabolites after 25% haemodilution with Fluosol (HF) or saline (HS).

	Treatment groups								
Parameters	CONT	0·5HF	24HF	48HF	72HF	0·5HS	24HS	48HS	72HS
Antipyrine (%)	4·4†	2·6	3·4	2·8	3·7	1·8*	5·8	5·1	4·1
	1·4	1·8	1·6	1·4	2·4	1·0	1·5	2·0	1·4
4-OH (%)	14·8	14·7	18·4	15·6	17·1	11·5	10·9	15·0	14·0
	2·7	2·2	3·9	6·1	8·4	2·1	2·8	4·2	3·5
3-OHME (%)	23·3	18·6*	17·0*	19·2	26·1	17·9	28·5	26·8	19·2
	2·0	2·0	3·3	4·8	11·5	5·3	8·1	5·4	5·1
k_u (min^{-1})	0·4	0·2*	0·1*	0·3	0·5	0·1*	0·3	0·3	0·3
($\times 10^3$)	0·1	0·1	0·09	0·2	0·3	0·07	0·07	0·1	0·2
4-OH k_f (min^{-1})	1·2	0·9*	0·8	1·3	2·1	0·8*	0·6*	0·8*	1·1
($\times 10^3$)	0·1	0·08	0·4	0·3	1·3	0·1	0·2	0·2	0·4
3-OHME k_f (min^{-1})	1·8	1·1*	0·7*	1·7	3·1	1·2	1·6	1·4	1·5
($\times 10^3$)	0·2	0·09	0·3	0·6	1·8	0·4	0·5	0·4	0·5
Cl_R	0·3	0·1*	0·1*	0·2	0·3	0·1*	0·2	0·2	0·3
(mL min^{-1} kg^{-1})	0·07	0·07	0·06	0·07	0·2	0·04	0·07	0·08	0·2
4-OH Cl_m	0·9	0·5*	0·8	0·9	1·2	0·7*	0·5*	0·7*	0·8
(mL min^{-1} kg^{-1})	0·08	0·1	0·4	0·2	1·0	0·1	0·2	0·07	0·3
3-OHME Cl_m	1·5	0·6*	0·7*	1·2	1·7	1·0	1·2	1·2	1·1
(mL min^{-1} kg^{-1})	0·2	0·1	0·3	0·4	1·3	0·4	0·5	0·2	0·5

† Mean with s.d.
* Significantly different from control ($P \leq 0{\cdot}05$).

Table 4. Averaged disposition of urinary antipyrine and metabolites after 50% haemodilution with Fluosol (HF) or saline (HS).

	Treatment groups								
Parameters	CONT	0·5HF	24HF	48HF	72HF	0·5HS	24HS	48HS	72HS
Antipyrine (%)	4·4†	2·8	3·3	1·1*	3·0	1·4*	1·4*	2·5*	1·5*
	1·4	0·9	0·4	0·4	1·1	0·4	0·5	0·5	0·8
4-OH (%)	14·8	10·4	12·4	9·3*	14·1	12·2	12·3	14·0	10·4
	2·7	6·0	2·2	1·6	1·9	1·7	1·8	2·5	4·3
3-OHME (%)	23·3	21·8	16·6*	45·2*	37·9	17·2*	30·2	33·3	17·7
	2·0	5·9	2·6	10·4	17·3	3·6	9·1	10·3	6·7
k_u (min^{-1})	0·4	0·2*	0·4	0·1*	0·3	0·1*	0·1*	0·06*	0·1*
($\times 10^3$)	0·1	0·07	0·2	0·06	0·08	0·05	0·03	0·02	0·03
4-OH k_f (min^{-1})	1·2	0·5*	1·3	1·0	1·4	0·9	1·2	0·6*	0·4*
($\times 10^3$)	0·1	0·3	0·3	0·1	0·5	0·3	0·1	0·08	0·1
3-OHME k_f (min^{-1})	1·8	1·2	1·9	5·1*	3·9*	1·3	3·0*	1·5	0·7*
($\times 10^3$)	0·2	0·6	1·0	1·8	2·1	0·5	0·7	0·4	0·3
Cl_R	0·3	0·2	0·2	0·2	0·1*	0·1*	0·2	0·1*	0·06*
(mL min^{-1} kg^{-1})	0·07	0·1	0·09	0·08	0·02	0·04	0·1	0·03	0·04
4-OH Cl_m	0·9	0·6*	0·6	1·2	0·7	0·9	1·7*	0·6*	0·4*
(mL min^{-1} kg^{-1})	0·08	0·3	0·3	0·4	0·3	0·3	0·4	0·2	0·2
3-OHME Cl_m	1·5	1·5	0·7*	6·0*	1·7	1·2	4·2*	1·3	0·6*
(mL min^{-1} kg^{-1})	0·2	1·1	0·4	2·6	0·7	0·4	2·0	0·1	0·3

† Mean with s.d.
* Significantly different from control ($P \leq 0.05$).

Tables 1 and 2 show the antipyrine pharmacokinetic parameters following 25 or 50% haemodilution, respectively. Tables 3 and 4 report the urinary excretion data corresponding to the plasma data. 25% Fluosol haemodilution initially increased the antipyrine $t_{\frac{1}{2}}$ (0·5 h, 24 h), but was significantly decreased at 72 h. 50% Fluosol haemodilution produced a similar effect on antipyrine $t_{\frac{1}{2}}$, although the decline in the parameter was seen after 24 h. As expected, the changes in antipyrine K_{el} inversely followed the pattern of the $t_{\frac{1}{2}}$ parameter. The antipyrine apparent volume of distribution (V_d) was significantly decreased at 0·5 and 72 h after 25% Fluosol haemodilution and 24 and 72 h after 50% Fluosol haemodilution. But V_d was significantly increased at 0·5 and 48 h after 50% haemodilution. Antipyrine clearance was significantly decreased at 0·5 and 24 h after 25% Fluosol haemodilution, while only the 48 h group was significantly increased after 50% haemodilution.

Antipyrine clearance was significantly decreased for 48 h after 25% saline haemodilution, and appeared to return to the control value after 72 h. After 50% haemodilution, clearance was increased at 24 h, then decreased below control values at 72 h. V_d was not altered in any saline-haemodiluted group except 24 h after 50% haemodilution.

25% Fluosol haemodilution decreased the urinary excretion rate constant of antipyrine (k_u) and the formation rate constants (k_f) of 4-OH and 3-OHME for 24 h. These parameters had returned to control values at 48 h and tended to be higher than control at 72 h, though not significantly different. After 50% Fluosol haemodilution, a similar pattern was evident with the return to control values occurring earlier (24 h), and with significant increases in 3-OHME seen at 48 and 72 h. 25% saline haemodilution decreased 4-OH formation for 48 h. After 50% saline haemodilution, antipyrine urinary excretion was decreased for 72 h. After a significant increase in the 4-OH and 3-OHME formation rate constants, the constants were significantly decreased at 48 and 72 h.

3-ME was not found to be a major urinary metabolite in the study. Only 7% of the animals excreted the metabolite in any detectable amount (assay sensitivity limit = 2 $\mu g\ mL^{-1}$), and the metabolite accounted for less than 5% of the dose. The metabolite has accounted for 7 to 21% of the dose in other reports (Danhof et al 1979b; Rhodes & Houston 1983).

Discussion

Investigations have shown that animals can survive moderate haemodilution with Fluosol without supplemental oxygen. A 71% Fluosol exchange without oxygen has been reported (Kemner et al 1984b). Rats in the present study underwent approximately a 25 or 50% haemodilution with Fluosol or saline without supplemental oxygen. Although most rats lost weight after haemodilution, long term studies have shown that haemodiluted animals grow comparably with unexchanged animals (Watanabe et al 1979).

Haematocrits were reduced approximately 25 or 50% after haemodilution, and the levels remained depressed through 72 h. Previous studies have shown that the haematocrit remained depressed longer after a one-third haemodilution compared with a near total exchange (Zucali et al 1979; 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).

The initial decrease and then an increased antipyrine clearance after haemodilution 25% and 50% with Fluosol is consistent with an induced enhancement of microsomal enzymatic activity. Since 50% haemodilution caused less of a decline but more of an increase in the clearance values compared with 25% haemodilution, it appears that more Fluosol present in the body caused a greater enhancement. The increased potency of the haemodilution 50% with Fluosol could be a dose dependent effect and/or the consequence of PVCs remaining longer in the animal since the elimination of these compounds has been shown to decrease with increasing dosage (Yokoyama et al 1982).

Haemodilution 25% with Fluosol decreased the antipyrine clearance by decreasing the formation of both 4-OH and 3-OHME. 4-OH formation has been shown to be dependent upon the 3-methylcholanthrene induced cytochrome P-448 activity, while 3-OHME formation is thought to depend on uninduced cytochrome P450 (Danhof et al 1979b; Teunissen et al 1983). Therefore, 25% haemodilution appears to briefly inhibit cytochrome P-450. The same effect on 4-OH and 3-OHME formation was not evident after the haemodilution 50% with Fluosol. Instead, 3-OHME formation was significantly enhanced at 48 and 72 h, suggesting that the haemodilution 50% with Fluosol may only induce cytochrome P-450, and has little influence on cytochrome P-448.

Enzyme inducers often produce inhibition before induction (Kato et al 1964). The potential enhancing agents in Fluosol could be one or both of the perfluorochemicals (PFCs) and/or Pluronic F-68 since all three are taken up by the liver (Lowe & Bollands 1985; Willcox et al 1978). However, the extent of liver uptake and retention of Pluronic F-68 appears to be much less than that of PFCs. Several PFC compounds have been shown to form an enzyme substrate complex with cytochrome P450 (Geyer 1982) which is one requirement of enzyme inducers (Greim 1981).

The long hepatic retention time of the PFCs could explain their ability both to inhibit and to enhance the enzymatic activity after a single exposure. Fluosol is a commercially prepared PFC emulsion containing two PFCs, perfluorodecalin and perfluorotripropylamine. The intravascular half-life of the two PFCs in Fluosol is approximately 10 to 13 h in the rat at dosages used in this study, and is 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 half-life of perfluorotripropylamine in the tissues is greater than 60 days (Geyer 1982; Yokoyama et al 1984; Mitsuno et al 1984).

The decrease in the antipyrine clearance in various groups after 25 or 50% saline haemodilution cannot be seen as a direct inhibition of microsomal enzymatic activity by sodium chloride. One possible explanation might be that saline haemodilution decreases hepatic oxygenation, thereby compromising the microsomal enzyme activity. One report (Zucali et al 1979) did show that plasma erythropoietin concentrations increased immediately after a plasma haemodilution to a haematocrit of 15%, which would indicate that hypoxia was present. The oxygen transport capacity of blood reaches a maximum when the haematocrit is 30 and is 90% of control values at a haematocrit of 20 (Goslinga 1984); these two haematocrits are equivalent to the animals 25 and 50% haemodiluted in the present study. Hepatic extraction of oxygen also increases in haemodiluted states (Lutz et al 1979). Therefore, it is unlikely that a deficiency in hepatic oxygenation is responsible for the decreased antipyrine metabolism, and the data suggests that some other mechanism may be responsible for the altered antipyrine clearance.

Antipyrine has been found to have a hepatic extraction ratio of 0·01 in the rat (Rane et al 1977). As such, its hepatic clearance (Cl_H) would be expected to be primarily dependent upon the unbound plasma free fraction (f_u) and the intrinsic unbound clearance of the liver (Cl_{INT}) in the relationship (Øie & Benet 1980):

$$Cl_H = f_u\ Cl_{INT}$$

It is expected that haemodilution will initially reduce the plasma proteins which would lead to an increased f_u and a corresponding increase in Cl_H if Cl_{INT} remains unchanged. After 25 or 50% haemodilution with either Fluosol or saline, total plasma protein and plasma albumin did not change in any group (unpublished data). Although f_u was not determined in this study, antipyrine is only 13% plasma protein bound in the rat (Soda & Levy 1975), suggesting that if an increase in f_u should occur, the influence on Cl_H would be minimal.

Changes in the hepatic Cl_{INT} may thus play a major role in the metabolism of antipyrine after haemodilution. It has 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 haemodilution (Miller et al 1979; Lutz et al 1982). A decreased number of hepatocytes could decrease the antipyrine clearance by decreasing Cl_{INT}. The presence of the PFC particles in the hepatocytes could alter the sinusoidal plasma membrane permeability in either a positive or negative manner. Whether changes in the sinusoidal plasma membrane permeability after Fluosol occur at all or occur at the percentages of haemodilution used in this study cannot be determined from the present study. It is also difficult to see that saline haemodilution could have the same influence on the number and size of hepatocytes, and changes in membrane permeability that Fluosol haemodilution produces.

A change in hepatic Cl_{INT} might occur if the hepatic microcirculation distribution is changed as the result of haemodilution. Such redistributions have been reported to occur in hepatic (Kessler & Messmer 1975) and cardiac tissue (Buckberg & Brazier 1975; Kessler et al 1983), perfused liver tissue (Ohyanagi et al 1983), and have been suggested to occur in pulmonary tissue (Nishimura et al 1983). The "intact hepatocyte" hypothesis (Øie & Benet 1980) states that an apparent reduction in the number of functional hepatocytes results when blood flow is shunted within or away from the liver. Thus if blood is diverted from areas of high functional hepatocyte density to low density areas, the Cl_{INT} may be altered. A change in Cl_{INT} could occur even without decreasing the number of hepatocytes if the drug is carried into hepatic areas that have different capacities and/or capabilities for metabolism. Furthermore, the possibility does exist that Fluosol and saline would produce different redistribution patterns, and that the extent of the redistribution might be dose-dependent. The antipyrine metabolite data after saline haemodilution shows that 25% haemodilution inhibited only 4-OH formation, while 50% haemodilution inhibited both 4-OH and 3-OHME formation. An inherent assumption in this proposed mechanism is that the hepatic microcirculation redistribution would be sustained at least 72 h. As the original work on hepatic redistributions (Kessler & Messmer 1975) indicated, the redistributions occurred immediately after haemodilution. There is no literature that determined the redistribution pattern for several days after haemodilution.

Antipyrine's apparent volume of distribution (V_d) is equal to the total body water volume (Stevenson 1977). Following both 25 and 50% saline haemodilution, V_d was not different from the respective control except at 24 h after 50% haemodilution. However, following 25% Fluosol haemodilution, V_d was significantly reduced approximately 35% at 0·5 and 72 h. Following 50% Fluosol hemodilution, V_d alternated between values higher than and less than the control values. Taken at face value, the data would suggest that the total body water volume decreases at select times after haemodilution with Fluosol. A more likely explanation is that several factors are influencing V_d, and that these factors do seem to be predominant after Fluosol haemodilution and only in drugs with significant hepatic elimination.

It has been reported that Fluosol haemodilution in rats caused a one-third reduction in the circulating blood volume after 3 h, and the blood volume remained depressed for 24 h (Matsumoto et al 1977; Watanabe et al 1979). In anaesthetized rats, hypovolaemia was produced as water transfers from the vascular to the interstitial fluid (Fournier et al 1980), but the driving force for the water transfer was not determined. There is no reported information on whether the hypovolaemia persists longer than 24 h, or if saline haemodilution would cause a similar effect.

The possible roles of plasma protein binding and hepatic microcirculation redistributions in antipyrine metabolism have been mentioned. Changes in plasma protein binding are known to have an effect on V_d (Gibaldi & McNamara 1978). As total plasma protein and plasma albumin did not differ in any haemodiluted group, V_d is not being influenced by protein binding. If redistributions in the body's microcirculation are responsible for the changes in V_d, then these redistributions would need to follow a cyclic or bimodal pattern after haemodilution. There is no literature that would indicate that the redistributions follow such a pattern.

Thus it appears that antipyrine metabolism and volume of distribution in the rat are dependent upon both the extent of haemodilution and haemodiluent used. Changes in the antipyrine clearance were accompanied by parallel changes in the 4-OH and 3-OHME metabolic clearance. The antipyrine V_d, when reduced, was always decreased approximately 35% regardless of the extent of haemodilution, and was reduced only in Fluosol haemodiluted animals. Several factors may be influencing V_d after Fluosol haemodilution, such as hypovolaemia or changes in microcirculation distributions. These results suggest that conventional dosing regimens for agents which undergo extensive hepatic metabolism may not be applicble in patients haemodiluted with Fluosol.

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References

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

Bizot, W. B., Rink, R. D. (1985) Effects of exchange transfusion with perfluorochemical emulsions on hepatic oxygen supply and blood flow in the rat. Experientia 41: 1127–1129

Branch, R., Shand, D., Wilkinson, G., Nies, A. (1974) Increased clearance of antipyrine and d-propranolol after phenobarbital treatment in the monkey. J. Clin. Invest. 53: 1101–1107

Buckberg, G., Brazier, J. (1975) Coronary blood flow and cardiac function during hemodilution. In: Messmer, K., Schmid-Schonbein, H. (eds) Intentional Hemodilution. Karger, Basel, pp 173–189

Danhof, M., de Groot-van der Vis, E., Breimer, D. D. (1979a) Assay of antipyrine and its primary metabolites in plasma, saliva, and urine by high-performance liquid chromatography and some preliminary results in man. Pharmacology 18: 210–223

Danhof, M., Krom, D. P., Breimer, D. D. (1979b) Studies on the different metabolic pathways of antipyrine in rats: Influence of phenobarbital and 3-methylcholanthrene treatment. Xenobiotica 11: 695–702

Fournier, G., Barrois, V., Girard, C., Robert, D., Foulletier, L. (1980) Total replacement of blood by an emulsion of Fluorocarbon in the rat-water extravasation as a cause of failure. Experientia 36: 568–569

Geyer, R. P. (1982) Recent development in research with perfluorochemical artificial blood substitutes. 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 19–29

Gibaldi, M., McNamara, P. J. (1978) Apparent volumes of distributions and drug binding to plasma proteins. Eur. J. Clin. Pharmacol. 13: 373–378

Goslinga, H. (1984) Lowering the viscosity with the aid of hemodilution. In: Blood Viscosity and Shock: The Role of Hemodilution, Hemoconcentration and Defibrination. Springer-Verlag, New York, pp 34–45

Greim, H. (1981) An overview of the phenomena of enzyme induction and inhibition: Their relevance to drug action and drug interactions. In: Jenner, P., Testa, B. (eds) Concepts in Drug Metabolism, Part B. Marcel Dekker, New York, pp 219–263

Griffeth, L. K., Rosen, G. M., Rauckman, E. J. (1984) Effects of model traumatic injury on hepatic drug metabolism in the rat. III. Differential responses of cytochrome P-450 subpopulations. Drug. Metab. Dispos. 12: 588–595

Kato, R., Chiesara, E., Vassanelli, P. (1964) Further studies on the inhibition and stimulation of microsomal drug-metabolizing enzymes of rat liver by various compounds. Biochem. Pharmacol. 13: 69–83

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

Kemner, J. M., Snodgrass, W. R., Worley, S. E., Hodges, G. R., Melethil, S., Hignite, C. E., Tschanz, C. (1984b) 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

Kessler, M., Messmer, K. (1975) Tissue oxygenation during hemodilution. In: Messmer, K., Schmid-Schonbein, H. (eds) Intentional Hemodilution. Karger, Basel, pp 16–33

Kessler, M., Vogel, H., Gunther, H., Harrison, D. K., Hoper, J. (1983) Local oxygen supply of the myocardium after extreme hemodilution with Fluosol-DA. 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 237–248

Lowe, K. C., Bollands, A. D. (1985) Physiological effects of perfluorochemical blood substitutes. Med. Lab. Sci. 42: 367–375

Lutz, J., Metzenauer, P. (1980) Effects of potential blood substitutes (perfluorochemicals) on rat liver and spleen. Pflugers Arch. 387: 175–181

Lutz, J., Wagner, M. (1984) Recovery from pentobarbital-induced sleep after administration of perfluorinated blood substitutes. Artif. Organs 8: 41–43

Lutz, J., Bauml, M., Schulze, H. G. (1979) Comparison of Fluosol-DA 35% with DA 20% in the circulation of the liver. In: Naito, R. (ed.) Proceedings of the Fourth International Symposium on Perfluorochemical Blood Substitutes. Excerpta Medica, Amsterdam, pp 123–135

Lutz, J., Metzenauer, P., Kunz, E., Heine, W. D. (1982) Cellular responses after use of perfluorinated blood substitutes. 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

Matsumoto, T., Watanabe, M., Hamano, T., Hanada, S., Suyama T., Naito, R. (1977) Role of oncotic agents in saving effects of perfluorochemical emulsion in hemodilution. Chem. Pharm. Bull. 25: 2163–2171

Matsumoto, J., Bianche, J., Thompson, R., Sharp, C., Andresen, B., Ng, K., Gerber, N. (1983) Disposition of phenytoin in rats treated with Fluosol-43, a perfluorochemical artificial blood substitute. Proc. West. Pharmacol. Soc. 26: 403–407

Miller, M. L., Moore, R. E., Clark, L. C. (1979) Morphology and morphometry of the liver after infusion of perfluorochemical emulsions: An assessment of 19 selected compounds. 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) Clinical studies of a perfluorochemical whole blood substitute (Fluosol-DA): Summary of 186 cases. Ann. Surg. 195: 60–69

Mitsuno, T., Ohyanagi, H., Yokoyama, K. (1984) Development of a perfluorochemical emulsion as a blood gas carrier. Artif. Organs 8: 25–33

Nishimura, N., Sugi, T., Hiranuma, N. (1983) The effect of Fluosol-DA on pulmonary vessels. 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 283–289

Ohyanagi, H., Matsui, E., Saitoh, Y., Hagihara, B. (1983) The effect of Fluosol(TM)-DA 20% FDA on microcirculation in isolated liver perfusion. 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 448

Øie, S., Benet, L. Z. (1980) Altered drug disposition in disease states. Ann. Reports Med. Chem. 15: 277–287

Rane, A., Wilkinson, G. R., Shand, D. G. (1977) Prediction of hepatic extraction ratio from in vitro measurement of intrinsic clearance. J. Pharmacol. Exp. Ther. 200: 421–424

Rhodes, J. C., Houston, J. B. (1983) Antipyrine metabolite kinetics in phenobarbital and β-naphthoflavone-induced rats. Drug Metab. Dispos. 11: 131–136

Shrewsbury, R. P. (1987) Effect of Fluosol-DA hemodilution on the kinetics of hepatically eliminated drugs. Res. Comm. Chem. Path. Pharmacol. 55: 375–396

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

Shrewsbury, R. P., Lewis, L. M., Oliver, S. R. (1987a) Effect of moderate haemodilution with Fluosol-DA or normal saline on low-dose phenytoin and (±)-5-(4-hydroxyphenyl)-5-phenylhydantoin kinetics. Ibid. 39: 349–356

Shrewsbury, R. P., Lewis, L. M., S. R., Womble, C. L. (1987b) Effect of moderate haemodilution with Fluosol-DA or normal saline on indocyanine green and (+)-propranolol kinetics. Ibid. 39: 592–598

Soda, D. M., Levy, G. (1975) Inhibition of drug metabolism by hydroxylated metabolites: Cross-inhibition and specificity. J. Pharm. Sci. 64: 1928–1931

Stevenson, I. H. (1977) Factors influencing antipyrine elimination. Br. J. Clin. Pharmacol. 4: 261–265

Teunissen, M. W. E., Meerburg-van der Torren, J. E., Vermeulen, N. P. E., Breimer, D. D. (1983) Automated high-performance liquid chromatographic determination of antipyrine and its main metabolites in plasma, saliva, and urine, including 4,4'-dihydroxyantipyrine. J. Chromatog. 278: 367–378

Tremper, K. K., Friedman, A. E., Levine, E. M., Lapin, R., Camarillo, D. (1982) The preoperative treatment of severely anemic patients with a perfluorochemical oxygen-transport fluid, Fluosol-DA. N. Engl. J. Med. 307: 277–283

Watanabe, M., Hanada, S., Yano, K., Yokoyama, K., Suyama, T., Naito, R. (1979) Long-term survival in rats severely exchanged-transfused with Fluosol-DA. In: Naito, R. (ed.) Proceedings of the Fourth International Symposium on Perfluorochemical Blood Substitutes. Excerpta Medica, Amsterdam, pp 347–358

Willcox, M. L., Newman, M. M., Paton, B. C. (1978) A study of labeled Pluronic F-68 after intravenous injection into the dog. J. Surg. Res. 25: 349–356

Wilson, V. L., Larson, R. E., Moldowan, M. J. (1982) A non-invasive method for the study of hepatic drug metabolism in rodents: Antineoplastic drug effects on antipyrine metabolism in mice. Chem.-Biol. Interactions 40: 159–168

Yokoyama, K., Watanabe, M., Naito, R. (1982) Retention of perfluorochemical (PFC's) in blood of human recipients after infusion of Fluosol-DA 20%. In: Frey, R., Beisbarth, H., and 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., Ohyanagi, H., Yoichi, S. (1984) A perfluorochemical emulsion as an oxygen carrier. Artif. Organs 8: 34–40

Zucali, J. R., Mirand, E. A., Gordon, A. S. (1979) Erythropoiesis and artificial blood substitution with a perfluorocarbon-polyol. J. Lab. Clin. Med. 94: 742–746