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Short Communications

Effects of exchange transfusion with perfluorochemical emulsions on hepatic oxygen supply and blood flow in the rat[1]

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Summary. Exchange-transfusion to hematocrit 20 with isotonic perfluorochemical (PFC) emulsions containing 3% hydroxyethylstarch (HES) in rats breathing 100% oxygen produced significant reductions of hepatic PO_2 and blood flow in comparison to rats hemodiluted with isotonic 3% or 6% HES solution. The results indicate that PFC and/or emulsifiers were associated with adverse effects on liver blood supply.
Key words. Perfluorochemical; hemodilution; hepatic PO_2; hepatic blood flow.

Isotonic iso-oncotic emulsions of perfluorochemicals have been studied as potential blood supplements and resuscitation fluids by virtue of their capacity to dissolve larger amounts of oxygen than solely aqueous solutions[3]. Two formulations (Green Cross Corp., Japan) have received particular attention: Fluosol-43, containing perfluorotributylamine (20% w/v), and Fluosol-DA, containing perfluorodecalin (14% w/v) and perfluorotripropylamine (6% w/v). Pluronic F-68, a nonionic detergent, is used as an emulsifier. Prior to use the emulsion is combined with hydroxyethylstarch and electrolytes. Fluosol-43 is restricted to investigations in animals due to long retention of the perfluorochemical in tissues such as liver and spleen. By contrast, the perfluorochemicals of Fluosol-DA are eliminated from the body over a period of weeks[3]. Fluosol-DA has been tested clinically in Japan with no major adverse effects reported[4].

Although clinical application of Fluosol-DA has been conducted on a restricted basis in the United States, in cases where patients refuse blood or blood products[5], there are questions about effectiveness[6] and concerns about adverse microcirculatory reactions[7,8]. Moreover, there is little information on the effects of perfluorochemical hemodiluents on tissue PO_2. The investigation reported here was undertaken to determine the relationship between changes, if any, of hepatic oxygen supply and blood flow attendant to hemodilution with perfluorochemicals in rats.

Materials and methods. Animal preparation and hemodilution procedure. Male Sprague-Dawley rats weighing 200–325 g were anesthetized by i.p. injection of pentobarbital sodium (40 mg/kg). PE-50 polyethylene cannulas were placed in the left common carotid artery and left external jugular vein. Rats breathed 100% oxygen. Following control measurements as described below, the cannulated animals were subjected to one of four treatments:

Perfluorochemical (PFC) hemodiluted. Animals in this group were exchange-transfused with either Fluosol-43 or Fluosol-DA (Green Cross Corp., Japan) prepared fresh for each rat. Hemodilution was conducted at the rate of 1.9 ml/min by simultaneous withdrawal of blood from the arterial cannula and infusion of diluent via the venous cannula until a hematocrit approximating 20 was reached. Additional volumes of diluent were added as needed to replace blood volume lost during sampling and to maintain mean arterial pressure throughout the experiment.

Hydroxyethylstarch (HES) controls. Using the same hemodilution procedure, animals were exchange-transfused to a similar hematocrit with an aqueous solution of 3% HES and electrolytes identical to that in which the PFC emulsions were mixed.

Additional control animals were exchanged with Hespan (American Critical Care), a 6% solution of HES in 0.9% NaCl.

Unexchanged, operated controls. Animals were prepared identically to those undergoing hemodilution. No exchange-transfusion was performed.

Measurements. In all animals colonic temperature was monitored throughout the procedure, and body temperature was maintained as necessary with a warming lamp. Systemic arterial pressure and heart rate were monitored continuously (Physiograph) via the arterial cannula.

Hepatic PO_2. Hepatic oxygen availability was estimated within 15–30 min after completion of hemodilution by surface measurement of tissue PO_2 using a multicathode probe[9]. The procedure was as follows: with the rat supine the ventral abdominal wall was incised along the midline and the edges retracted. The multicathode, containing six 25-µm diameter platinum wires and suspended by a flexible multistrand cable, was lowered onto the liver surface. Capillary adhesion secured the measuring end of the instrument to the tissue surface and allowed the polyelectrode to follow the movements of the liver associated with breathing. With the unit in place the PO_2 of tissue subjacent to each electrode was measured for 5 s before the polyelectrode was moved to a neighboring area and the measurements repeated. Repetition of this procedure during a 10-min interval provided sufficient tissue PO_2 values (80–100) to construct a freqency distribution curve, or PO_2 histogram, which characterizes tissue oxygen supply[10]. To insure the accuracy of PO_2 measurements, the electrodes were calibrated in known concentrations of oxygen at 37 °C both before and after each series of measurements.

Hepatic blood flow and cellular function. Following hepatic PO_2 measurements, liver blood flow and hepatocellular function were assessed by determination of clearance rates for indocyanine green dye (ICG; CardioGreen) in dosages of 5 or 30 mg/kg. The rate of clearance for the lower concentration is largely a reflection of hepatic blood flow while that of the higher dose is largely a reflection of hepatocellular function[11].

Determination of ICG clearance was conducted as follows. Solutions of ICG were prepared fresh for each experiment by reconstitution of the dye with 0.9% NaCl solution. After removal of 100 µl of blood from the arterial cannula for a reference sample, the ICG dose (only one dose per rat) was injected (< 10 s) and the cannula flushed with 0.9% NaCl solution. The dye was allowed to circulate for 1 min, then 5 100-µl samples were drawn at intervals of 30 s (for 5 mg/kg dose) or 60 s (for 30 mg/kg) until six samples were taken. Between sampling the volume removed was replaced via the venous cannula with the appropriate diluent or 0.9% NaCl in the unexchanged group. The next step in the standard procedure would have been to dilute the samples in isotonic 1% albumin solution, centrifuge, and measure absorbances in the supernatant. However, it was found that centrifugation of samples from PFC diluted rats resulted in deposition of both PFC and ICG in the pellet, suggesting that some ICG became attached to the PFC particles. Affinity studies demonstrated that ICG is soluble in PFC, and exists in a reversible equilibrium between the PFC and the surrounding aqueous medium. Consequently, it was necessary to modify the standard procedure by diluting samples from all groups in 1% albumin and 0.2% sodium bicarbonate to hemolyze the red cells. No centrifugaiton was employed. ICG concentrations were read against a standard curve at 805 nm on a Leitz MQ3 spectrophotometer. Tests showed that hemolysis and the presence of PFC did not materially affect the accuracy of dye concentration measurements. Absorbance values were plotted on semilogarithmic paper, and ICG half-times ($t_{½}$) were determined from the slopes of the curves. Clearance rate (v) was determined by the formula $v = 0.693\ (dose)/t_{½}$[11].

Evaluation of data. Comparisons between groups for PO_2 and $t_{½}$ data were examined for statistical significance using Student's t-test.

Results. The effects of hemodilution with Fluosol-DA or Fluosol-43 on the various parameters were similar. For convenience, the data have been combined.

Exchange-transfusion: volumes and cardiovascular parameters. The data on the volumes exchanged in the hemodilution procedure are presented in table 1; the volumes of PFC and HES infused did not differ significantly. Effects on hematocrit and basic cardiovascular parameters are illustrated in table 2. Hypervolemic exchange-transfusion to hematocrit approximating 20 with PFC or HES diluents did not cause significant changes in systemic arterial pressure or heart rate.

Hepatic PO_2. The results of hepatic PO_2 measurements are shown in the figure. Exchange-transfusion with either diluent was associated with a significant decrease in hepatic PO_2. Moreover, mean PO_2 in PFC hemodiluted rats was reduced to a level significantly below that of the HES group. PFC hemodiluted animals also had a higher percentage of PO_2 values in the range of 0–5 mm Hg.

ICG clearance. The results of ICG clearance measurements are shown in table 3. It can be seen that at the 5 mg/kg dose values for $t_{½}$ and velocity show significant ($p < 0.001$) variations between HES, PFC, and unexchanged groups. At the 30 mg/kg dose clearance values for PFC and unexchanged groups no longer show a significant difference. Half times at 30 mg/kg for the HES group remain significantly different ($p < 0.01$) from those for the other two groups.

Discussion. The data indicate that exchange-transfusion to hematocrit 20 in rats with PFC emulsions containing 3% HES and electrolytes was associated with a profound decrease of hepatic oxygen availability. Since the reduction of hepatic PO_2 was significantly larger than when isotonic 3% or 6% HES solutions

Table 1. Volume relationships in exchange-transfusion with hydroxyethylstarch (HES) or perfluorochemical (PFC) emulsion in rats

Group	Volume ml/kg		Ratio
	Infused	Withdrawn	
HES*	58.6 ± 2.4	47.6 ± 1.5	1.24:1
PFC**	63.4 ± 2.0	49.9 ± 1.1	1.27:1

Values expressed as mean ± SE. *Isotonic 3% or 6% hydroxyethylstarch solution. **Fluosol-DA or Fluosol-43.

Table 2. Effects of exchange-transfusion with hydroxyethylstarch (HES) or perfluorochemical (PFC) emulsions on hematocrit (HCT), heart rate (HR) and mean systemic arterial pressure (MSAP)

Group	Control period			Post-exchange period		
	HCT (%)	HR (beats/min)	MSAP (mm Hg)	HCT (%)	HR (beats/min)	MSAP (mm Hg)
HES*	46.1 ± 0.7	426 ± 5	134 ± 3	19.3 ± 0.4	463 ± 7	122 ± 5
PFC**	46.8 ± 0.6	425 ± 8	131 ± 3	21.4 ± 0.8	456 ± 9	115 ± 5
CON***	44.9 ± 0.7	421 ± 13	128 ± 5	45.8 ± 0.7	424 ± 11	122 ± 2

Values expressed as mean ± SE. *Isotonic 3% or 6% hydroxyethylstarch solution. **Fluosol-DA or Fluosol-43. ***Control, no exchange-transfusion, second measurements taken 20–30 min after control period.

Table 3. Values for indocyanine green (ICG) half-time ($t_{½}$) and clearance velocity in rats exchange-transfused with hydroxyethylstarch (HES) or perfluorochemical (PFC) emulsions

Group	Dose ICG (mg/kg)	$t_{½}$ (min)	Velocity (mg/kg/min)
HES[a]	5	1.2 ± 0.1[d]	2.9 ± 0.2[d]
PFC[b]		2.7 ± 0.2[d]	1.3 ± 0.1[d]
CON[c]		1.7 ± 0.1[d]	2.0 ± 0.1[d]
HES	30	5.1 ± 0.4[e]	4.1 ± 0.3[d]
PFC		7.7 ± 0.07	2.8 ± 0.3
CON		8.2 ± 0.06	2.6 ± 0.2

Values expressed as mean ± SE. [a]Isotonic 3% or 6% hydroxyethylstarch solution. [b]Fluosol-DA or Fluosol-43. [c]Control, no exchange-transfusion. [d]Significant difference in comparison to other values at same dose ($p < 0.001$). [e]Significant difference in comparison to other values at same dose ($p < 0.01$).

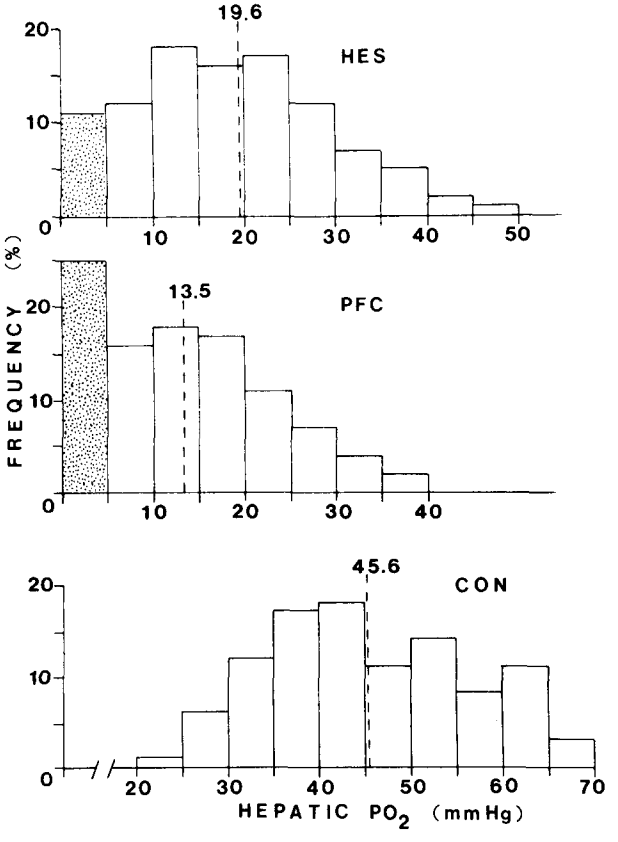
were used as diluents, it is apparent that the presence of PFC and/or emulsifiers had a deleterious effect on oxygen delivery. The concomitant slower clearance velocity of low dose ICG in the PFC groups suggests impaired hepatic blood flow[11], which is interpreted as the basis for the decline of hepatic PO_2. Consideration must be given to the possibility that longer clearance times in the PFC group were in part a reflection of the affinity of ICG for PFC, as noted in 'materials and methods'. However, affinity studies showed the combination to be reversible, and clearance times for high dose ICG were not slowed by the presence of PFC. Moreover, the hepatic PO_2 estimations by themselves provide circumstantial evidence of restricted liver blood flow.

The mechanism by which PFC emulsions affect hepatic blood flow is unclear; however, vasoactive effects have been observed in other studies. When PFC was infused into rabbits microvascular changes consisting of margination of leukocytes, endothelial damage, extravasation of blood cells, and slowed flow were observed in the omentum[7]. Margination of leukocytes and thrombocytopenia have been reported in rats[12,13]. In dogs infusion of PFC emulsions may cause a precipitous decrease of blood pressure[14]. Adverse hemodynamic effects may stem from complement activation by PFC emulsions, as reported in rabbits and humans[8].

In contrast to differing clearance velocities between PFC rats and controls for low dose ICG, those for high dose ICG were similar. Near saturation concentrations of ICG are used to assess hepatocellular function[11]; consequently, the results indicate that neither the PFC emulsion nor the relatively low hepatic PO_2 impaired the capability of hepatocytes to clear ICG, suggesting that PFC emulsions per se had no untoward effect on the function of liver cells. Further consideration of the ICG data shows that clearance velocities for both low and high dose ICG in the HES group were significantly faster than controls, as well as the PFC group. This may reflect increased functional capacity in response to more rapid blood flow. Several factors might account for stimulation of flow in HES diluted rats. Decreased hematocrit reduces blood viscosity particularly across low pressure gradients as in liver[15]. Given the variations of blood flow in different zones of liver acini[16], hemodilution and decreased viscosity may increase flow and hence, ICG delivery, in sinusoids which ordinarily have relatively slow flow.

Despite apparent augmentation of hepatic blood flow, liver PO_2 in HES diluted animals was significantly lower than in controls, albeit not as low as in the PFC group. Presumably the increase of tissue blood flow was not of sufficient degree to offset the decreased oxygen carrying capacity of blood with 20% hematocrit. The reductions of hepatic PO_2 in both PFC and HES were not attributable to hypotension since systemic arterial pressure remained similar to that in unexchanged controls. Similarly, hypoxemia was not a factor; a previous study[17] demonstrated that arterial PO_2 exceeds 500 mm Hg when rats breath 100%, as in this study.

In summary, the results of the present study indicate that the perfluorochemical emulsion component of Fluosol-DA and Fluosol-43 was associated with a significant reduction of hepatic blood flow during exchange-transfusion in rats. Our data do not provide clarification regarding the source of disturbance in hepatic blood flow, i.e. whether it stems from events upstream in the splanchnic circulation or whether it is largely a reflection of events within the liver. Reduced microcirculation is cited as the basis for the fact that hepatic oxygen supply declined to levels below those of animals hemodiluted with 3% or 6% hydroxyethylstarch solution. The effect of PFC hemodilution on tissue PO_2 in rats contrasts with the high values reported in the liver, pancreas, kidney, and skeletal muscle of dogs[18]. It is possible that species differences of sensitivity to PFC exist. Variations in factors such as complement activation may have a major role in determining the capability of perfluorochemicals to enhance tissue oxygen supply.



The frequency distribution of hepatic PO_2 in rats exchange transfused to hematocrit 20 with 3% or 6% hydroxyethylstarch (HES) or perfluorchemical (PFC) emulsions Fluosol-DA or Fluosol-43. Controls (CON) were not hemodiluted. All rats breathed 100% oxygen. Vertical dashed line indicates mean PO_2. $p < 0.01$ for PFC vs HES. $p < 0.005$ for PFC and HES vs CON.

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