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PARTICLE GROWTH OF FLUOROCARBON EMULSIONS IN THE LIVER AND SPLEEN

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Perfluorocarbon (PFC) oxygen transport emulsions appear to be chemically inert *in-vivo*. Accumulation of material in the liver and spleen is easily detected, and results in a weight increase in these organs (Lutz and Metzenauer 1980; Lowe 1988). The droplets appear to be present in clumps, normally termed 'foamy vesicles' due to their electronmicrographic appearance. The droplets are released from the liver into the blood, from where the PFC vapour is excreted via the lungs (Tsuda et al 1988). The relevance of this transient tissue uptake to the overall excretion process is not known. In order to understand the fate of the droplets *in-vivo* in more detail, we have re-extracted them from the liver and spleen and measured their particle size. This is possible because of their stability to freezing and centrifuging, during which very little coalescence takes place.

PFC emulsion was prepared as described previously (Sharma et al 1988), and contained 30%w/v perfluorodecalin (FDC), 2% soya oil and 4% Pluronic F-68 (PF68) in water. The diameter of the droplets in this emulsion was 0.22 μm. A similar dose of Fluosol DA 20% (Green Cross Corporation, Japan) was also used (droplet diameter 0.2 μm). Emulsions were injected intraperitoneally into male Wistar rats at a dose of 10 ml kg^{-1}. After 72 h, rats were killed and the liver and spleen dissected out. Approximately 0.1g of spleen or 0.5g of liver was gently macerated in a tissue grinder with 5ml of 4% PF68 solution and the resulting homogenate centrifuged at 3000 rpm for 30 min. The supernatant was discarded, and the cell pellet carefully rinsed away to provide a clean perfluorocarbon pellet, which was resuspended in 1 ml of 4% PF68 solution. Microscopy confirmed the sole presence of liquid droplets with no tissue fragments. These were then sized using a Malvern Mastersizer.

The sizes of the recovered droplets are given in Table 1. All the droplets recovered had a mean diameter of 1-10 μm, with no submicrometre droplets detectable. A control experiment, in which the FDC emulsion was mixed with tissue homogenate and recovered, provided a mean droplet diameter of 0.25μm, indicating that the recovery procedure did not cause major droplet coalescence.

Table 1.

	PFC emulsion	Fluosol DA
Liver	3.38 ± 0.09μm	4.97 ± 0.37μm
Spleen	6.85 ± 1.06μm	6.13 ± 0.74μm

The droplets recovered from the liver were generally smaller than those recovered from the spleen. There appeared to be no difference in the diameters of the droplets from the two formulations, although more emulsion was recovered from Fluosol-treated tissues than the FDC emulsion-treated tissues. The statistical significance of the differences was difficult to assess with such small samples, since the particle sizer was working at the limits of its sensitivity, It is clear, however, that considerable coalescence occurred in the liver and spleen. Coalescence almost certainly potentiates the excretion of PFC's via the lungs, since droplets released from the liver and spleen are sufficiently large to lodge in the lung capillary bed. The original PFC droplets are too small to accumulate in this manner. These results suggest that transient uptake by the liver and spleen is an essential part of the PFC excretion process.

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