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In Vitro Depression of Tissue Oxygen Uptake by Liquid Fluorocarbon Prepared as Artificial Blood (39668)

W. I. ROSENBLUM, R. M. NAVARI, J. E. LEVASSEUR, AND J. L. PATTERSON, JR.

Departments of Pathology (Neuropathology) and Medicine, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298

Liquid fluorocarbons carry O_2 and CO_2 in large amounts. They give O_2 off to body tissues and carry CO_2 away. These properties have led to the suggestion that emulsions of fluorocarbon in physiologic salt solution might function as artificial blood. Workers, using fluorocarbon emulsions as whole blood infusions or transfusions, or employing these emulsions as perfusates for isolated organs (1-3), have equilibrated the emulsion with very high concentrations of O_2. These concentrations generally approach 100% (760 Torr). High O_2 levels have been used because the solubility of O_2 in fluorocarbon is dependent upon the partial pressure, and it was thought that high O_2 concentrations would permit one to take full advantage of the O_2 carrying capacity of the fluorocarbon. However, no work has been published defining the lower limits of ambient O_2 at which fluorocarbon emulsions might be efficacious in comparison with aqueous plasma expanders which carry very little O_2. Moreover, little data has been published concerning the effects of fluorocarbon emulsions on tissue *in vitro*, in systems much simpler than those employing whole animals or whole organs. The studies described below employ a system to measure tissue oxygen consumption (Q_{O_2}) and to test the effects of a reduction in ambient O_2 on tissue Q_{O_2}, when fluorocarbon emulsions are used as the suspending medium in the tissue bath. We find that fluorocarbon emulsions can depress Q_{O_2}, and the depression is more marked as P_{O_2} is reduced below 760 Torr. In seeking an explanation for this effect, we find that O_2 diffusion falls in fluorocarbon as the P_{O_2} falls, but this phenomenon is not of sufficient magnitude to explain the effect on Q_{O_2} in these particular experiments.

Materials and methods. Microdissection technique. A microdissection technique (4, 5) was used to obtain respiratory bronchioles from adult male Sprague-Dawley rats (220-225 g), and the oxygen consumption of the bronchioles was measured using a Cartesian diver microrespirometer. Dissection of the lungs to obtain the respiratory bronchioles was performed with a stereomicroscope containing a calibrated reticle in the eyepiece. After determination of the diameter of the respiratory bronchiole *in situ*, a tubular length was excised free of adjacent pulmonary tissue and cut into tubular segments, each approximately 500 μm in length, 300-400 μm in diameter, and 50-100 μg in dry weight. The tissue segments were transferred to a Petri dish filled with a physiological salt solution of pH equal to 7.30±0.05 (KCl 2.8 m*M*, $CaCl_2$ 1 m*M*, NaCl 142 m*M*, NaH_2PO_4 11 m*M*, glucose 1.1 m*M*) or a fluorocarbon/physiological salt solution emulsion. The bronchioles consisted of mucosa and smooth muscle and were free of cartilage.

Fluorocarbon emulsions. The fluorocarbon emulsions utilized either one of two fluorocarbons (FC): FC-47 (perfluorotributylamine or CF_3 $(CF_2CF_2CF_2)_3N$, produced by 3M Corporation) and PP-5 (perfluorodecalin or $C_{10}F_{18}$, produced by I.S.C. Ltd., England).

The emulsions were made in one of three ways: (a) they were emulsified by sonication (3) in a salt solution and used after sonication; (b) they were sonicated in distilled water, the emulsion deionized in a mixed bed resin, and the physiologic salts then added; (c) they were homogenized in physiologic salt solution in a Gaulin Homogenizer at 3000 lbs/in^2. The end point of emulsification for the sonicates was defined by passage through a 0.8 μm filter. The homogenates also were passed through the fil-

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ter, but in addition, their optical density (OD) was measured and homogenization was not stopped until the OD had fallen to a plateau. Fluoride ion concentration was measured in all emulsions with a specific ion electrode. It varied from less than 0.5 ppm to 3.2 ppm. Results were independent of the fluoride ion concentration. The emulsions employed 33% to 40% FC on a w/v basis. The FC was added to Ringers Lactate Solution containing, per liter, 154 mEq Na, 4 mEq K, 3 mEq Ca, 109 mEq Cl, 28 mEq lactate. To this solution was added 48 g/L of pluronic F-68, a nonionic detergent required for emulsification. The detergent also adds osmotic strength. The pH was adjusted with Tris-buffer to 7.30±0.10. The osmolarity of the emulsions was 272-292 mOsm/L, and the results were independent of osmolarity over the stated range.

Cartesian diver microrespirometer. The Cartesian Diver is a well-established method (4, 6–8) for measuring the Q_{O_2} of minute tissue samples. The samples are placed in a small, floating chamber (the diver) whose buoyancy is altered as the tissue consumes oxygen. As O_2 is consumed, the pressure within the system is manipulated in order to maintain the diver at a fixed level in the flotation medium. The pressure required to float the diver was plotted against elapsed time, and the change in the amount of oxygen in the diver was calculated from the first derivative of the Ideal Gas Law with respect to time to give the oxygen consumption of the tissue. All samples showed a constant change in pressure with time over a 5-h period, indicating a steady state condition.

Bronchial tissue, approximately 0.25 μl in vol, was suspended in 0.5 μl of the suspending medium (physiologic salt solution or fluorocarbon emulsion) and placed in the diver. Above the tissue and its substrate was a gas bubble which provided buoyancy and was the source of oxygen. The composition of the gas in the gas bubble could be varied (4), and experiments were conducted with mixtures ranging from 5% O_2, 95% N_2 to 95 O_2, 5% N_2. A KOH seal in the diver neck absorbed all the CO_2 produced by the tissue (4, 6–8), and there was no change in the pH of the incubation medium measured before and after each Q_{O_2} determination.

As distinguished from the existing macrorespirometers, which are equipped with a stirring device, the diver respirometer and in fact all microrespirometers are able to function without stirring, the movement of the gases being considered fast enough to secure equilibrium and adequate mixing in systems of sufficiently small dimensions. Linderstrom-Lang (8) has presented both experimental data and a mathematical analysis which give the limitations of diver dimensions for various gas–liquid systems to ensure adequate mixing and a rapid approach to the attainment of equilibrium. The diver employed in this study was well within the size limitations to ensure adequate mixing in the experiments performed.

In order to ensure that the replacement of salt solution by fluorocarbon emulsion did not alter the mixing properties of the system by creating unstirred layers adjacent to the tissue, the time required for O_2 utilization in the diver to reach equilibrium was recorded. If unstirred layers were present, the time to achieve equilibrium would be altered and/or equilibrium might never be reached. Equilibrium is achieved when the fall in partial pressure of O_2 per unit time becomes constant, and measurements are begun after equilibrium is reached. For rat bronchiole rings of identical size (350 μm diameter) from the same animal, the time to reach equilibrium was rapid and essentially the same in the control physiological salt solution (8.43±0.38 min) and the fluorocarbon emulsions of different dispersed phase (fluorocarbon) concentrations (8.33±0.37) min, 25% (w/v); 8.37±0.31 min, 33% (w/v); 8.57±0.33 min, 40% (w/v).

Diaphragm diffusion cell. The oxygen diffusivity in each of the fluorocarbon emulsions used in the diver was measured using a steady state diaphragm cell which consisted of two compartments separated by a fritted glass diaphragm.

A detailed description of the diaphragm cell can be found elsewhere (9, 10). This experimental technique has been used extensively with good success by other investigators for gaseous diffusion in pure liquids (10), single protein solutions (11), and multiple polymer and protein solutions (12).

Results. Effect of fluorocarbon emulsions on Q_{O_2}. Table I shows that the Q_{O_2} was not

TABLE I. BRONCHIOLE Q_{O_2} IN CARTESIAN DIVER MICRORESPIROMETER.

Tissue suspending medium	Fluorocarbon (w/v%)	Q_{O_2}[a] (μL/mg dry wt/hr)	N[c]	Gas mixture (vol %)	
				O_2	N_2
Physiologic Salt Solution	–	3.43 ± 0.17	39	20	80
Physiologic Salt Solution	–	2.63 ± 0.11[b]	5	5	95
Physiologic Salt Solution	–	3.51 ± 0.21	5	10	90
Physiologic Salt Solution	–	3.49 ± 0.11	11	95	5
Ringers lactate with F-68 detergent	–	3.39 ± 0.17	5	20	80
Fluorocarbon (PP-5) Emulsion	40	1.69 ± 0.13[b]	10	20	80
Fluorocarbon (PP-5) Emulsion	40	2.71 ± 0.13[b]	5	95	5
Fluorocarbon (FC-47) Emulsion	34	1.99 ± 0.13[b]	5	20	80
Fluorocarbon (FC-47) Emulsion	33	2.69 ± 0.15[b]	10	35	65
Fluorocarbon (FC-47) Emulsion	33	2.98 ± 0.10[b]	10	50	50
Fluorocarbon (FC-47) Emulsion	34	3.23 ± 0.29	10	95	5

[a] Mean ± standard deviation.
[b] Significantly different from control physiologic salt solution at 20% O_2, 80% N_2. $P < 0.01$, Student's t test.
[c] N is the number of bronchioles tested.

depressed in the presence of 20% O_2 and a control solution of Ringers Lactate with detergent. However, fluorocarbon emulsified in the lactate solution, did significantly depress the Q_{O_2} at 20% O_2. As the ambient O_2 was raised, the Q_{O_2} increased, until, in an atmosphere of 95% O_2, the Q_{O_2} in FC-47 emulions was not significantly different from control. Note also that increasing the O_2 in the atmosphere had no effect on the Q_{O_2} of control preparations. Thus, the diffusion of O_2 through the control tissue was not a factor limiting its Q_{O_2}. Moreover, the hourly Q_{O_2} was constant over the entire period of observations (5 hr) for all tissue samples. Although this check is performed to guard against a leaky diver, it also indicates that for tissue in the FC emulsions, suppression of Q_{O_2} was rapid, and thereafter was nonprogressive.

O_2 diffusion in fluorocarbons and fluorocarbon emulsions. The Q_{O_2} data could be explained if O_2 diffusion became impaired in fluorocarbons as O_2 tension fell. To test this hypothesis, we measured the apparent O_2 diffusion coefficient in pure fluorocarbons and in fluorocarbon emulsions, using the steady state diaphragm cell (Methods). Table II shows that the apparent diffusion coefficient for O_2 in the pure fluorocarbons was high at P_{O_2} = 760 torr. The coefficient obtained at P_{O_2} = 760 torr was similar to that for three other pure fluorocarbons published in the literature (13). We have not encountered data at lower P_{O_2}, and the measurements of this study show a decreasing O_2 diffusion coefficient with a fall in P_{O_2}. These observations are compatible with the implications of Zander's data (14) showing an increasing fall in solubility coefficient as P_{O_2} fell. The latter implies some chemical binding of O_2 by FC, with an increasing number of chemical binding sites available for O_2 as P_{O_2} falls. These sites would be available to "trap" O_2 and retard its movement through the fluorocarbon down the gradient of O_2 tension. Measures of diffusion in the diaphragm cell would not distinguish this phenomenon from others impairing movement of O_2 molecules. Since the diffusion coefficient will be dependent upon the P_{O_2}, we feel the term "apparent" is best used in describing the coefficient.

Discussion. Although movement of O_2 through FC is impaired as P_{O_2} falls, this change in diffusion coefficient fails to provide a viable explanation for the fall in Q_{O_2} which we observed accompanying the decline in P_{O_2}. Table II shows that at 150 torr, the apparent diffusion coefficient of O_2 in FC and in FC emulsions is higher than the coefficient in H_2O. In addition, we have calculated the flux of O_2 through the diffusion cell in ml O_2/sec and found that even at a P_{O_2} of 50 torr, the flux would exceed that through H_2O at similar P_{O_2}. This is because total flux is proportional both to the diffusion coefficient and to the O_2 content of the fluid. FC carries more O_2 than water, and this factor overrides the reduction in the diffusion coefficient.

Since reduced diffusion of O_2 cannot ex-

TABLE II. O_2 DIFFUSION IN PURE FLUOROCARBONS AND IN FLUOROCARBON EMULSION AT 37°.

Substance	P_{O_2}(mmHg)	D[a]	C_{O_2}* (ml O_2/ml)[b]	N (ml O_2/sec)[c]
FC-47	760	5.79 ± 0.20	0.362 ± 0.019	76.21
FC-47	150	4.97 ± 0.19	0.062 ± 0.003	11.20
FC-47	100	4.35 ± 0.18	0.037 ± 0.002	5.85
FC-47	50	1.93 ± 0.10	0.015 ± 0.001	1.05
25% FC-47 Emulsion	760	3.03 ± 0.09	0.093 ± 0.005	10.27
25% FC-47 Emulsion	150	2.84 ± 0.10	0.018 ± 0.001	1.81
33% FC-47 Emulsion	760	3.21 ± 0.14	0.117 ± 0.008	13.69
33% FC-47 Emulsion	150	2.96 ± 0.09	0.020 ± 0.001	2.15
H_2O	760	2.75 ± 0.12	0.024 ± 0.001	2.38
H_2O	150	2.75 ± 0.12	0.005 ± 0.002	0.46
H_2O	100	2.75 ± 0.12	0.003 ± 0.0001	0.31
H_2O	50	2.75 ± 0.12	0.002 ± 0.0001	0.16

[a] D = diffusion coefficient, mean ± standard deviation × 10^{-5} cm²/sec.
[b] C_{O_2}* = O_2 content when equilibrated with the stated P_{O_2}, Mean ± Standard Deviation.
[c] N = O_2 "flux", $N = (DA/L)(C_{O_2}^* - C_{O_2})$ where A/L is an apparatus constant, and C_{O_2} is zero.

plain the observed depression of Q_{O_2}, we must seek elsewhere for an explanation of the findings. There exists the possibility that there is a toxic effect of FC on the tissue. Fluorocarbons are known to displace structures within liver cells and cells of the reticuloendothelial system (3) and mitochondrial swelling has been reported both *in vivo* (3) and *in vitro* (15).

In vitro mitochondrial respiration has been depressed by FC (15). However, the *in vivo* observations of mitochondrial swelling (though not the displacement of cellular organelles) could conceivably be artifact, while the *in vitro* effects on mitochondria have been reported only for chlorinated, gaseous fluorocarbons rather than the liquids used here. Moreover, bronchial smooth muscle is the predominant tissue in our bronchial rings, and we are not aware of published data describing toxic effects of fluorocarbons on smooth muscle. We have, in fact, found only a single report (16) of Q_{O_2} measurements in th presence of FC, *in vitro*. This report fails to demonstrate depression of Q_{O_2}, but the data are peculiar in several respects. Tissue slices from liver, heart, and kidney were employed and were apparently so thick that diffusion of O_2 was limiting and basal Q_{O_2} values were only 1% of those found here. The authors were able to raise Q_{O_2} twenty fold simply by increasing the ambient P_{O_2}. An identical effect of increasing P_{O_2} was found both in the presence or absence of FC. One would expect that Q_{O_2} would improve much more in the presence of FC since O_2 delivery to the tissue should be increased. Perhaps the slices were so thick that FC at their surface had little impact on their overall Q_{O_2}.

Whatever the reason for the effect we have observed, our data indicate that the depression of Q_{O_2} is gradually overcome as concentrations of O_2 approach 100%. However, the degree of Q_{O_2} depression and the capacity of increasing O_2 tensions to overcome this depression may be dependent upon the fluorocarbon used and its concentration. Our data show that the effect of a PP-5 emulsion on Q_{O_2} was not completely reversed by 95% O_2, while the effects of a slightly lower concentration of FC-47 were reversed by 95% O_2.

Since O_2 tensions approaching 100% are frequently employed with FC systems, this may explain the failure of some workers (1, 2) to observe the effect we have seen after infusion or transfusion of whole animals or organs with large amounts of FC. However, the relationship of our *in vitro* data to an *in vivo* situation remains open to question, since *total* exchange of blood for FC emulsion (2) has been reported in rats with no ill effects (but again at high P_{O_2}). One of us (WIR) has attempted such exchange transfusions in rhesus monkeys, and has succeeded in replacing 50% of the blood at ambient (21%) O_2 tensions, and 75% of the blood while the monkey breathed 100% O_2. Whether the phenomena reported here will provide a barrier to continued development of FC emulsions as a blood replacement remains to be seen. They do suggest that ambient O_2 levels approaching 100% will

have to be maintained as long as high FC levels are circulating.

Sumary. The present studies show for the first time that, *in vitro*, fluorocarbon emulsions depress O_2 uptake of tissue. This depression can be overcome totally or in part by use of O_2 levels approaching 100% of the ambient atmosphere. Our studies also show that the diffusion coefficient for O_2 in fluorocarbons and in fluorocarbon emulsions is dependent upon the O_2 tension and falls as P_{O_2} falls. However, due to the high O_2 content of fluorocarbon, the O_2 flux in fluorocarbons is higher than that in control solutions. Therefore, the fall in diffusion coefficient cannot account for depression of O_2 uptake in these studies. The cause of the depressed Q_{O_2} remains unknown, but toxicity of fluorocarbon should be considered. The data suggest that if fluorocarbon is to be an efficacious blood substitute, high levels of inspired O_2 will have to be maintained as long as high fluorocarbon levels exist in the circulatory system.

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