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Accelerated Removal of Platelets during Perfusion of Isolated Lungs with Perfluoro Erythrocyte Substitute (40641)[1]

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The high solubility of oxygen and carbon dioxide in some liquid fluorochemicals (perfluoro compounds) has permitted their use as liquid breathing media for small animals (1). Certain of these compounds, when dispersed in an aqueous medium into particles smaller than erythrocytes, have successfully carried out the gas transport functions of the erythrocyte. This was first demonstrated in experiments with an isolated perfused rat brain (2) when it was found that the metabolic and electrical activity (EEG) of the brain were maintained as well by perfusion with emulsified fluorochemical as with erythrocytes, but that perfusion with plasma alone resulted in rapid loss of electrical activity (3). Subsequently, the ability of the dispersed perfluoro compounds to deliver oxygen to tissues of intact animals was demonstrated (4–6). Although it was clear that the dispersed perfluoro compound transported oxygen *in vivo*, its injection into the circulating blood was sometimes followed by derangements of circulatory and pulmonary function (7, 8). Intravenous injection of emulsified fluorochemical caused a large decrease in the number of circulating blood platelets (9) and in some animals, there were gross lesions in the lungs and a marked increase in resistance to flow of blood through the lungs (10). Since these results might be due to an action of fluorochemical on lungs and/or blood platelets, we studied the effects of adding emulsified fluorochemical to the fluid perfusing isolated lungs when blood platelets were either present or absent from the fluid. Our results indicate that the fluorochemical did not have a direct, deleterious effect on the lungs. The accelerated removal of platelets from the perfusion fluid which occurred suggests that there was an interaction between fluorochemical particles and platelets which resulted in deposition of platelets in the lungs.

Materials and methods. Emulsions of FC-80 (perfluorobutyltetrahydrofurane, 3M Co.) were freshly prepared for each experiment by sonication in an 8% solution of bovine serum albumin (Fraction V, Sigma Chem. Co.) in Krebs–Ringer bicarbonate buffer, as previously described (3). The final concentration of the fluorochemical in the emulsion was approximately 40% (v/v), which is about 80% (w/v) since the density of the fluorochemical is nearly 2. The particles were 1 μm in diameter and the pH of the emulsion was 7.35.

Male guinea pigs (Hartley strain) or male rats (Sprague–Dawley strain) weighing about 300 g, were anesthetized with pentobarbital and ventilated mechanically through a tracheostomy. After a catheter was placed in the pulmonary artery, the lungs were removed and suspended in a water-jacketed chamber maintained at 37°. The pulmonary artery was perfused at 10 ml/min with recirculating fluid consisting of 4% bovine serum albumin and 5 m*M* glucose in Krebs–Ringer bicarbonate buffer (pH 7.4) which was aerated with 5% CO_2 in oxygen. The lungs were ventilated with the same gas mixture at a rate of 80 per min and a tidal volume of 2.5 ml. The lung preparation, with its connecting tubing, was suspended by three wires from an analytical balance (Mettler Model P163) and weighed continuously. A weight change of 10 mg (0.5% of the weight of the lungs) was reliably detectable. The pressures required for lung ventilation and perfusion were monitored by strain gauge transducers and recorded continuously. The pH of the perfusion fluid was monitored continuously with a flow cuvette

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and glass electrode and the pH was maintained between 7.35 and 7.45 by addition of either sodium hydroxide or hydrochloric acid solution. The technique of perfusion has been described previously (11, 12).

Blood platelets were obtained from human blood which had been collected in 3.5% sodium citrate (1 to 9 vol of blood). Human, rather than rat, platelets were used because we were interested specifically in the interaction of human platelets with emulsified fluorochemical. Our control experiments indicated that human platelets and rat lungs were apparently compatible. Platelet-rich plasma was prepared by centrifugation of the blood in plastic tubes at 200*g* for 10 min at room temperature. Platelets were counted by the method of Brecher and Cronkite (13).

Results. In one group of experiments, isolated lungs were perfused with fluid which contained no platelets. Emulsified FC-80 was absent (controls) or was added. The pressure required for tidal ventilation was approximately 5 to 7 cm of water and the mean perfusion pressure (with reference to the top of the lung) was 12 to 18 cm of water. Lung preparations perfused for 60–75 min without fluorochemical (controls) showed no significant changes in perfusion pressure or ventilation pressure and the weight of the lungs increased less than 5% of the initial weight. In five experiments (two rat and three guinea pig lung preparations) emulsified FC-80 was added to the perfusion fluid to give a concentration in the range of 5–10% (v/v). This concentration of emulsified fluorochemical when injected into the circulation of intact animals, has been previously shown to cause definite changes in pulmonary circulation and in the lungs (8, 9). In each of the five perfusion experiments, the addition of dispersed FC-80 caused no change in ventilation pressure and only a slight increase (<2 cm water) in perfusion pressure. Similar slight increases in perfusion pressure were observed when a suspension of washed erythrocytes was added to the perfusion fluid, suggesting that the slightly increased pressure was due to increased viscosity of the perfusion fluid rather than to a change in the lung. The weight of the lungs increased by less than 5% after 60–75 min of perfusion.

In another group of experiments, rat lungs were perfused in the same way but 30 min before addition of emulsified FC-80, human platelet-rich plasma was added to the perfusion fluid to yield a concentration of approximately 200,000 per mm^3. In these experiments, as in those done without platelets, there were no significant changes in pressures required for ventilation or pulmonary perfusion after addition of FC-80 emulsion to the perfusion fluid. Platelet counts were made at intervals after addition of platelet-rich plasma and after the subsequent addition of emulsified FC-80. In two control experiments, the emulsified FC-80 was omitted. In two additional control experiments both platelets and emulsified FC-80 were added but the lungs were omitted from the circuit in order to evaluate the loss of platelets during perfusion by deposition outside the lungs. The changes in the platelet counts during 60 min of perfusion are shown in Fig. 1. In two experiments without the lungs in the circuit, an average of 94% of the original platelets remained after 30 min. This suggests that a slow rate of platelet removal during lung perfusion was due primarily to platelet dis-

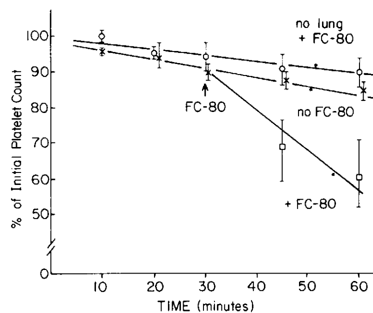

FIG. 1. The effects of emulsified fluorochemical on the rate of removal of human platelets from the fluid perfusing isolated rat lungs. The volume of recirculating perfusion fluid was 80 ml. In all experiments, 5 ml of a human platelet suspension was added at time zero, yielding an initial platelet count of approximately 200,000 per mm^3. The curve designations are: no lung + FC-80 for control experiments where the lungs were omitted from the perfusion circuit but FC-80 was added at the time indicated; no FC-80 for control experiments where the perfusion circuit was complete but FC-80 was not added; +FC-80 for experiments where the perfusion circuit was complete and FC-80 was added at the time indicated. In the experiments with FC-80, 10 ml of an emulsion (40% v/v) was added and the concentration of FC-80 in the perfusion fluid after mixing was 8% (v/v). The values shown are the average and the range of values.

ruption or adherence to elements of the perfusion circuit, with only a small contribution due to the presence of the lung itself. The rate of platelet loss was not significantly changed with perfusion up to 60 min in the absence of FC-80 emulsion, nor by the addition of FC-80 emulsion in the absence of the lung. The rate of removal of platelets from the perfusion fluid was significantly accelerated at both 15 and 30 min after addition of the FC-80 emulsion when the lungs were present (Fig. 1).

Histologic examinations of rat lungs after 30 min of perfusion with the FC-80 emulsion showed slight perivascular "cuffing" with fluid similar to that observed in control lungs, but no other abnormalities were noted by light microscopy.

Discussion. In our experiments, the lungs were perfused at a constant rate and ventilated at a constant tidal volume. Therefore, changes in the lung tissue, its airways, or its circulation would have led to changes in ventilation or perfusion pressure as previously shown by the large changes in pressures after infusion of 5-hydroxytryptamine (11) or with development of pulmonary edema (12). Therefore, the observed lack of change in perfusion and ventilation pressures after addition of the fluorochemical in the present study indicates that the pulmonary vascular resistance and mechanical properties of the lungs were essentially unchanged during the 60 min of perfusion. The absence of significant alveolar edema was also indicated by the absence of significant increase in lung weight and by histologic examination. These results suggest that the fluorochemical emulsion did not have a direct toxic effect on the isolated perfused lung. On the other hand, our results suggest that the emulsified fluorochemical interacted with blood platelets in a way that resulted in their increased rate of removal. The addition of emulsified FC-80 did not result in an increased rate of removal of platelets in the absence of the lung, suggesting that the platelets (aggregated and/or otherwise changed) were removed in the pulmonary circulation. The lesions which occur in the lungs of an intact animal after infusion of emulsified fluorochemical might be caused by a series of events which starts with pulmonary deposition of platelets. This possibility is supported by the observations (9, 10) of marked reductions in circulating platelets *in vivo* and significant changes in coagulation factors (9) in animals infused with fluorochemical emulsions. Although there was not an increase in pulmonary vascular resistance in the perfused lungs after addition of fluorochemical to the platelet-containing perfusion fluid, the difference between this situation and the intact animal may be due to the absence of coagulation factors in the fluid perfusing the isolated lungs.

Recent work (14) has shown that putting a coating of lipid on the emulsified particles prevents some of the effects of FC-80 on blood platelets. This finding may lead to the development of a fluorochemical emulsion which does not cause lesions in the lungs and is safe for use as a blood substitute in intact animals.

Summary. An emulsion of a fluorochemical (FC-80), which has been shown to function as a substitute for the erythrocyte in the transport of O_2 and CO_2, has been found to cause lung damage when infused intravenously in intact animals. Addition of this emulsion to the fluid perfusing rat and guinea pig isolated lung preparations caused no significant changes in the pressures required for ventilation and perfusion of the lungs at constant flow rates, or in the weight of the lungs. Addition of this emulsion after prior addition of human blood platelets, caused a large increase in the rate of removal of platelets from the perfusion fluid. These results indicate that the fluorochemical emulsion does not have a direct deleterious effect on the lungs but that an interaction between fluorochemical particles, blood platelets, and the lung occurs, which might lead to lung damage.

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