PERFLUOROCHEMICAL EMULSIONS AS BLOOD SUBSTITUTES

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ABSTRACT. Perfluorochemicals (PFC's) dissolve large volumes of oxygen (35-52 vol. %) and carbon dioxide (135-160 vol.%) and are chemically and biochemically inert. These properties have made them candidates for blood substitutes and other therapeutic applications requiring oxygen delivery to tissues. In this paper, the history of PFC's in medicine and the requirements for a successful commercial emulsion are briefly reviewed. The practical aspects and current status of the production of a safe, shelf stable, small particle size, high PFC content emulsion are reviewed and recent advances in the field such as new PFC's, novel surfactants, new clinical trials and recent regulatory approvals are presented.

1. Introduction

Whole blood serves a number of critical functions including delivery of oxygen to tissues, hemostasis, host defense, maintenance of ionic and protein balances, transport of nutrients and hormones, and the removal of metabolic waste products. However, since oxygen deprivation rapidly and irreversibly degrades the function of cells, tissues, and organs, leading to death, the most important function of blood is oxygen delivery to the tissues.

Historically, donated human blood has been the therapeutic of choice for acutely and chronically anemic patients. However, the use of donated blood is not without risks. Blood can be contaminated with an array of infectious agents including HIV, hepatitis virus, cytomegalovirus (CMV), Epstein- Barr virus, and *Brucella abortus*. While blood is currently screened for many pathogens including HIV, it is not possible to identify all pathogens nor to eliminate them with heat sterilization. Recent estimates place the risk of contracting non-A, non-B hepatitis at 7-10% of all patients transfused in the USA, with 50% of these patients developing chronic, active hepatitis (Haljamae and Rosenberg 1988).

Immunosuppression after transfusion of homologous blood is also a significant problem and has been linked with increased recurrence of cancer (Blumberg et al. 1985) and with an increased incidence of postoperative infection in certain types of surgical patients (Maetani et al. 1986). In addition to the health related risks inherent in blood usage, there are other limitations to the use of donated blood. Donor blood must be typed and cross-matched for each patient, resulting in a transfusion delay of at least 20 to 30 min. The use of type O negative blood to circumvent the typing and crossmatching time delay is not without risks.

Blood has a storage lifetime of only 42 days and it must be refrigerated. This makes blood unavailable in many of the situations in which it is most needed, such as in rural trauma incidents, in ambulances and helicopters, on battlefields and during civilian disasters. Complicating the storage lifetime is the fact that after blood has been stored for a few days, the erythrocyte depletes its 2,3-DPG and oxygen bound to hemoglobin in these cells becomes relatively unavailable. Complete restoration of 2,3-DPG content and oxygen delivery function requires about 12 hours in the circulation. Thus, transfusion of stored red cells does not immediately fulfill the oxygen transport function for which it was administered (Huetis et al. 1981).

The search for agents to replace the oxygen transport function of blood has been underway for over 50 years and has centered largely on three approaches: purified, hemoglobin derivatives, synthetic heme complexes and perfluorochemical (PFC)

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emulsions. Synthetic hemes have remained an academic curiosity while hemoglobin and PFC emulsions have been extensively studied. The goals of both PFC and hemoglobin research have been to develop a readily available, disease-free, shelf stable, safe, cost-effective, universal donor product that would be available when and where it was needed. While neither approach has yet achieved broad clinical success, enormous progress has been made with PFC emulsions over the last five years in understanding and overcoming obstacles to successful product introduction. New medical uses for oxygen transport agents have been identified and explored. Several PFC products are in various stages of preclinical and clinical evaluation. In late 1989, Fluosol™, developed and manufactured by the Green Cross Co. (Osaka, Japan), was approved as an oxygen transport agent for distal oxygenation of the myocardium during high risk coronary balloon angioplasty by the US FDA (Anonymous 1990).

The purpose of this chapter is to review the use of PFCs and their emulsions in medical oxygen transport and to highlight recent developments in the field.

2. History of Perfluorochemicals in Oxygen Transport

Fluorine, which is the most reactive and corrosive of all the halogens, forms the most stable single bonds to carbon known to chemists, with bond dissociation energies on the order of 116-122 Kcal/Mol compared to 102 Kcal/Mol for a typical carbon - hydrogen bond. This high bond strength translates into enormous molecular stability and chemical inertness, particularly when the carbon skeleton is completely fluorinated.

PFC's were first prepared in the 1930's in small quantities for research purposes from direct fluorination of carbon. However, the fact that fluorine was only available in gram quantities and could not be purchased at all until after World War II severely limited the scope of investigations of preparative methods for PFC's. During World War II, the Manhattan Project required the preparation of extremely inert solvents for separation of uranium isotopes via diffusion as their hexafluorides. This need served as the impetus to develop commercially useful methods of producing fluorine. Shortly thereafter fluorine became readily available electrochemically and methods of synthesis of perfluorinated compounds were developed resulting in the preparation of a wide variety of structurally diverse and commercially useful PFC's.

PFC's were found to have a number of interesting properties including chemical inertness, high gas solubility and diffusivity, and low toxicity. The only significant reactions of PFC's are pyrolysis and aromatization at temperatures around 350 to 550°C. Early studies of the fundamental physical properties of PFC's included measurements of gas solubilities, performed by Gjaldbaek and Hildebrand. They found that PFC's dissolved gases in accordance with Henry's law and remarkably, they could dissolve 10 to 20 times the gas dissolved by water (Gjaldbaek and Hildebrand, 1949).

Leland Clark galvanized medical research in the perfluorochemical field when he demonstrated that mice and young puppies submerged in liquid perfluorobutyltetrahydrofuran saturated with oxygen could derive their physiological oxygen requirements via the PFC in their lungs without toxicity (Clark and Gollan 1966). Within a year of Clark's publication, Henry Sloviter reported that PFCs could be rendered into a plasma compatible form by emulsification with bovine serum albumin in Kreb's Ringer bicarbonate (Sloviter and Kamimoto 1967). Using this emulsion, he was able to extend the electrical activity in isolated, perfused rat brains far longer when compared to rat erythrocytes. Geyer exchanged the blood of rats with an emulsion made from perfluorotributylamine, the surfactant pluronic F-68, and physiological salts. Animals survived on PFC "blood" at high oxygen tensions until sufficient regeneration of RBCs had occurred to support life on room air (Geyer et al. 1968, 1973). The rats developed normally and survived in apparent good health to the end of their normal life expectancy. These total exchange experiments were a graphic demonstration of both the efficacy and the

safety of PFCs. The fact that the rats survived with hematocrits as low as 3% demonstrated the physiological gas transport capabilities of PFCs. The safety of the emulsion was evident because serum albumin, immunoglobulins, clotting factors, platelets, leucocytes, and erythrocytes, which had all been removed as a consequence of the total exchange, all regenerated, indicating no damage to the liver or the marrow.

Subsequently, clinical research groups initiated animal studies and identified potential clinical applications for PFC oxygen transport agents in fields ranging from shock resuscitation and wound healing to heart attack and cancer therapy. Industrial research resulted in the preparation of the first clinically acceptable emulsion, Fluosol, by the Green Cross Co, (Osaka, Japan).

3. Characteristics Required of Perfluorochemicals for Medical Use

The pioneering work of Clark, Sloviter and Geyer allowed a clear definition of the requirements for a successful commercial oxygen transport agent based on PFC emulsions.

The PFC required two seemingly simple characteristics:

(A) Readily available in high purity - At the time interest arose in their medical applications, PFC's were exclusively used in industrial applications including vapor phase resoldering and as fire resistant lubricants. The purity requirements for industrial grade materials are significantly different than the specifications for a fluid for intravenous use. Many potential impurities in PFC's, particularly compounds bearing residual hydrogens and double bonds, are too reactive to be used intravenously.

(B) Satisfactory tissue residence times without toxicity - Geyer's work had shown that perfluorotributyl amine was sequestered in the liver and spleen of the treated rats for the duration of their lifetime. Lifetime sequestration of xenobiotics, particularly in the quantities required for oxygen transport agents, is rightfully considered medically unacceptable.

Using these guidelines, Clark (Clark et al. 1974), in the US, and Yokoyama (Yokoyama et al. 1975) in Japan screened over 100 PFC's searching for compounds which had the right combination of properties to form a stable emulsion, possess minimal side effects and which would leave the body in a reasonable amount of time. Of these only a handful received detailed study. Reiss's excellent 1978 review of PFC blood substitutes contains citations for each study for the reader interested in more historical detail (Reiss and LeBlanc, 1978).

The results of this research were the development of rather general, empirical rules for the physical properties of PFC's suitable for intravenous usage. In general, the rate at which a PFC is eliminated from the body was found to be related to its molecular weight, structure, vapor pressure and lipophilicity, with vapor pressure being perhaps the major determinant. Generally, the higher the vapor pressure, the more rapidly the PFC is eliminated from the body. However, the relationship of vapor pressure to organ clearance rate is not perfect, and structural changes and heteroatoms have a significant impact on tissue clearance rate regardless of vapor pressure. For example, perfluorodecalin, with a vapor pressure of 12 mmHg, was found to leave most organs in which it was sequestered with a $T_{1/2}$ of seven days. However, perfluoromethyldecalin, with a vapor pressure of 5-6 mm/hg, has an organ $T_{1/2}$ of 105 days while perfluorotripropylamine, with a vapor pressure of 20 mmHg, has an organ $T_{1/2}$ of 65 days; quite a difference in half-life for such minor changes in structure, molecular weight or vapor pressure! Addition of a heteroatom to a structure generally increases its organ $T_{1/2}$. For example, perfluoro-4-methyl-4-isopropylpentane has an organ $T_{1/2}$ of 11 days while a similar C-9 ether, perfluoro-4,4-dimethylbutylpropyl ether has a $T_{1/2}$ of 35 days (Reiss, 1984). Molecular weight can also be misleading as a predictor of tissue clearance time. For example, perfluorooctyl bromide has a molecular weight of 499 and an organ $T_{1/2}$ of 7 days (Liu, 1973) compared to

perfluoromethyldecalin with a molecular weight of 512 and an organ $T_{1/2}$ of 105 days. It is unlikely that this small difference in molecular weight could have such a profound impact on organ half-life, and suggests that molecular weight is not the key determinant of organ half-life *within* the structural range embraced by C8 to C12 PFC's. The critical solution temperature (CST), an index of PFC lipophilicity has been proposed as a predictor of tissue clearance rate (Moore and Clark, 1985). The CST is a measure of the temperature required for a PFC to dissolve in hexane. The data support the hypothesis that, within the range of C8 to C12, the molecules with a lower CST and thus a higher lipophilicity have lower tissue residence times. In the final analysis, the clearance rate depends upon a poorly understood and almost totally empirical relationship between structure, molecular weight range, vapor pressure and lipophilicity.

At the same time it was found that PFC's with vapor pressures above 30 mmHg were unacceptable because they caused pulmonary hyperinflation (an emphysema like condition without any visible tissue lesions) and subsequent lethality due to pulmonary complications (Yokoyama, Suyama and Naito, 1982). This result places an upper ceiling on the acceptable range of PFC vapor pressures. When this screening work was completed, it was clear that the structures of medically acceptable PFC's were fairly restricted between 8-12 carbons, were preferably more lipophilic and required vapor pressures between about 10-20 mmHg in order to be non-toxic and yet clear the body within a reasonable timeframe. While these studies narrowed the range of structures that might be physiologically acceptable, all structures within that range still had to be synthesized and tested individually for toxicity and clearance rate before conclusions about their medical utility could be drawn.

The net result of these studies was the recognition that only four of the PFC's synthesized and tested had the correct balance of organ retention time and safety necessary to be further considered for commercial development as medical oxygen transport agents (Table 1). These were perfluorodecalin, perfluoromethyladamantane, 1,2 bis(perfluorobutyl)-ethylene and perfluorooctyl bromide. Predictably, Clark was the first to discover the rapid transpiration rate of both perfluorodecalin and perfluoromethyladamantane (Clark, Wesseler, Miller and Kaplan, 1974). The organ clearance data for the liver and the spleen for perfluorodecalin compared to perfluorotributylamine shows that PFD leaves the organs in a dose dependent fashion and is largely gone from rat livers and spleens at the doses of two to eight g/kg in about two weeks, while perfluorotributylamine content does not decrease at all during the same time frame.

Table 1. Properties of Perfluorochemicals tested for medical oxygen transport

Perfluorochemical	MW	Vapor Pressure	Organ $T_{1/2}$(days)	CST (°C)	Toxicity
Perfluorodecalin	462	12	7	22	non-toxic
Perfluoromethyldecalin	512	6	105	50	non-toxic
Perfluorobutyltetrahydro-furan	416	58	na	28	toxic
Perfluorooctahydoindan	412	34	na	na	toxic
Perfluoro-4-methyl4-isopropylpentane	488	na	11	na	na
Perfluoro-4,4-dimethylbutyl propyl ether	504	na	35	na	na
Perfluorotributylamine	671	2	400	61	non-toxic
Perfluorotripropylamine	521	20	40 to 60	43	non-toxic
Perfluorodimethyladamantane	524	na	10 to 20	32	non-toxic
Perfluoro-1,3-dimethylcyclohexane	400	67	na	na	toxic
Perfluorooctyl Bromide	499	12	7	<0	non-toxic

4. Characteristics Required for Medical PFC Emulsions

The properties required for the PFC emulsion are much more extensive:
(A) Surfactant safety - The surfactant used in PFC emulsions must be biocompatible and extremely non-toxic.
(B) Good shelf-stability of the PFC emulsion - Quarantine times post manufacture coupled with distribution and shipping times mandate as long a shelf life as possible for an emulsion with a minimum shelf life of six months and a preferred shelf life of 18 months. Storage temperature is flexible for a pharmaceutical product but room temperature storage is cheapest and most convenient.
(C) *In vivo* emulsion stability - An emulsion must be stable to the ions, proteins and enzymes of the plasma in order not to undergo particle size growth. Growth of particles in the plasma could result in PFC emboli forming in the microcirculation, causing widespread tissue ischemia.
(D) Stable during terminal sterilization at 121°C - All large volume parenteral products sold in the US today are terminally sterilized at 121°C to insure complete reduction of any bioburden which may have been acquired in the processing of the product. Historically, this requirement has been a serious obstacle for PFC emulsions.
(E) High PFC content - In contrast to hemoglobin which has a sigmoidal oxygen delivery curve that is 97% saturated at normal ambient pO_2's of about 150 mmHg of oxygen, PFC emulsions carry oxygen in direct proportion to both the PFC content and the oxygen tension of the gaseous environment with which the emulsion is in contact. Since the

emulsion will be diluted upon infusion into the patient, the concentration of PFC in the emulsion needs to be as high as possible to insure sufficient circulating concentration of PFC in the patient.
(F) Acceptable particle size distribution (PSD) - The particle size of an emulsion plays an important role in its toxicity and organ distribution. Early investigators in the intravenous fat emulsion field discovered that large particles, in the size range 5-15μm, were sieved by the pulmonary circulation resulting in significant pulmonary toxicity.
(G) Viscosity compatible with whole blood - It is well established that perfusion and tissue oxygenation are inversely related to the viscosity of blood. As blood viscosity increases, the rate of perfusion falls and so does the effectiveness of tissue oxygenation. The viscosity of an emulsion must not increase the viscosity of the circulating blood to a point where the flow characteristics become significantly impaired and reduce perfusion.
(H) Intravenous half-life - The emulsion must remain in the circulation long enough to insure a therapeutically useful period of oxygen delivery.

5. Medical Oxygen Transport with Perfluorochemicals

The fact that neat PFC's dissolve large amounts of oxygen when in equilibrium with pure oxygen gas is well documented and the number of measurements made on PFC's since interest arose in their use as blood substitutes has lead to a large and structurally diverse database on their oxygen solubility properties. There have been a number of mechanisms proposed to explain the enormous solubility of gases in PFC's, including charge transfer interactions, structural sites in the PFC molecule itself and cavities in the bulk solution phase as a consequence of the very weak Van der Waal's attractions between molecules. The cavity proposal has received some support from NMR studies of the relaxation times of carbon and fluorine nuclei when oxygen is dissolved in PFC's (Hamza et al. 1981). Regardless of mechanisms, the oxygen content of PFC's is significant compared to other fluids.

Oxygen dissociation curves for PFC emulsions and hemoglobin are distinctly different. PFC oxygen content is linearly dependent upon the oxygen tension with which it is in equilibrium. Gases dissolved in PFC's behave like gases dissolved in water; that is, they are driven only by the environmental gradients. Oxygen moves into the PFC phase when environmental tensions are high and out when they are low. There is no chemical affinity of PFC's for oxygen. In contrast, hemoglobin exhibits a sigmoidal dependence upon oxygen tension and is 97% saturated at atmospheric oxygen tensions. Thus, oxygen tension has two important ramifications for PFC emulsions. First, they require supplemental oxygen to be achieve maximum oxygen content and second, they can exploit oxygen therapy far better than hemoglobin which is saturated at about 150 mm Hg of oxygen.

The oxygen content in PFC emulsions on thus depends directly on PFC content and oxygen tension: the more PFC in the emulsion, the greater the oxygen content of the resulting solution. This underscores the need for a high PFC content emulsion to insure an adequate circulating concentration of PFC in the patient resulting in adequate oxygen transport after dilution of the infused emulsion by the patient's systemic circulation.

The starting point for assessment of a PFC emulsion's oxygen transport capability is its oxygen content at various oxygen tensions *in vitro*. However, the ultimate utility of a PFC emulsion to transport oxygen therapeutically depends upon its contribution to oxygen delivery and ultimately to oxygen consumption. Oxygen delivery is the product of arterial oxygen content (CaO_2) and cardiac output (CO) (Equation 1):

$$QO_2 = CaO_2 \times CO \qquad (1)$$

The arterial oxygen content for blood containing PFC is the sum of the three phases carrying oxygen: $[O_2]aHb$, the oxygen content due to hemoglobin, $[O2]aPFC$, the oxygen content due to PFC and $[O2]aH2O$ (Equation 2).

$$CaO_2 = [O_2]aHb + [O_2]aPFC + [O_2]aH2O \quad (2)$$

To assess the fractional contribution of an emulsion to oxygen delivery requires that the arterial and venous contents of all three phases be known or calculated. The oxygen content due to hemoglobin can be measured using a co-oximeter or calculated using the hematocrit and the well accepted value of 1.34 cc O_2 per gram of hemoglobin. The contribution of the aqueous and PFC phases can be calculated from the blood gases, the Bunsen solubility coefficients for water and PFC and the fluorocrit [the fluorocrit (Fct) is the volume percent of PFC, not emulsion, in the whole blood]. By Henry's law, the arterial or venous oxygen content of the PFC phase depends upon the solubility coefficient (a_{PFC}) of the PFC and the arterial or venous blood gases as shown for arterial content in equation 3:

$$[O_2]aPFC = Fct \times PaO_2/760 \times a_{PFC} \quad (3)$$

The arterial or venous content of the aqueous phase can be calculated from the arterial and venous blood gases, the solubility coefficient for water and for the fluorocrit and hematocrit, as shown for the arterial aqueous content according to equation 4:

$$[O_2]aH2O = PaO_2/760 \times (1-Hct-Fct) \times a_{H2O} \quad (4)$$

These equations coupled with known solubility coefficients and a few measurements, available from the patient's chart, allow the investigator to determine the relative contribution of PFC's to the overall oxygen transport of a given patient. The equations may also be coupled with direct oxygen content measurements to determine solubility coefficients for any emulsion at any given temperature as Moss's group has done for Fluosol® (Rosen et al. 1985).

6. Medical Applications of Perfluorochemicals and Their Emulsions

Perfluorochemical emulsions have been investigated in a number of therapeutic roles including their use as blood substitutes, as therapy for ischemic heart and cerebral tissue, as a adjunct to balloon angioplasty, as cardioplegia for coronary bypass surgery, as imaging agents, as an adjunct to cancer radiation therapy and as a therapy for the bends. Most of these applications have been investigated in animal models and at least four indications have been the subject of clinical studies and one indication has received FDA clearance. This section will focus on the major indications in which sufficient animal and/or clinical data exist to evaluate the therapeutic value of PFCs.

6.1 BLOOD SUBSTITUTES

The most frequently investigated therapeutic application for PFC emulsions has been as an oxygen transport substitute for red blood cells. Red blood cell substitutes cover many possible, diverse indications including hemorrhagic shock resuscitation, restoration of blood oxygen content during elective and emergency surgical hemorrhage, priming pumps during coronary artery bypass and as an oxygen transport diluent for autologous donation preceding elective surgery.

Geyer's experiments with total exchanged rats provided the first substantial evidence that PFC emulsions could take the place of blood in providing the total oxygen requirements of an animal. Subsequently, exchange transfusions have been reported in dogs (Suyama et al. 1975) and monkeys (Ohyanagi et al. 1978; Rosenblum et al. 1985). Monkeys were exchanged transfused with either Fluosol or the plasma expander Hespan (HES) to a hematocrit of <2%. After six hours, the survivors were infused with autologous blood. All of the HES-exchanged monkeys died before the blood transfusion, while 8 of 10 monkeys given Fluosol survived to receive the blood transfusion, and six of the eight that received blood after six hours survived.

Okada and coworkers studied the use of PFCs in resuscitation of hemorrhagic shock in dogs (Okada et al. 1975). Arterial, venous and skeletal muscle oxygen tensions were significantly higher animals resuscitated with Fluosol DC than in the group resuscitated with Lactated Ringer's. Remarkably, the mixed venous oxygen tension of the Fluosol DC group was 78 mm Hg indicating that, in anesthetized, intubated dogs breathing 100% oxygen, the dog's oxygen requirements were largely being met by the PFC with almost no oxygen off-loading by hemoglobin (hemoglobin is greater than 90% saturated at 78 mm Hg).

Ohyanagi and Mitsuno (1975) compared shock resuscitation with PFC or low-molecular-weight dextran (LMD). In all cases, the PFC emulsion produced superior survival to the LMD resuscitation, and when combined with blood transfusions given six hours post resuscitation, 100% survival was observed. The data from these studies offer strong support that interim resuscitation by PFC emulsions in environments where blood is not immediately available followed by blood transfusion could make an enormous difference in survival of shock victims.

Makowski compared Fluosol and Fluosol DA-35 with 6% HES and whole blood in shock resuscitation (Makowski 1978). All four treatments restored mean arterial pressure and cardiac index and increased mean pulmonary artery pressure. Blood and both PFC emulsions restored arterial and venous oxygen contents, but HES did not. All treatments restored oxygen consumption immediately after infusion, however, the PFC emulsions increased oxygen consumption compared to HES or blood. Mixed venous oxygen tensions were also higher immediately after perfusion in the PFC-treated animals. In addition, Makowski found that while 80% of the shocked animals reinfused with whole blood or HES died, only 20% of the Fluosol treated animals succumbed. The increase in oxygen consumption indicated that PFC emulsions are superior at reducing the oxygen debt that accumulate as a consequence of hemorrhagic shock.

A more recent study by Elliott and coworkers compared Fluosol to lactated Ringer's (LR) in an dog resuscitation model. The authors concluded that Fluosol was effective in producing volume expansion, oxygen delivery, and oxygen consumption. They observed an increasing oxygen consumption at 60 min. post-infusion similar to previous work and which was statistically significantly greater at 24 hours post infusion than LR. The authors also found that the PFC contributed as much as 40% to the animals' overall oxygen consumption in the one-hour period post shock (Elliott et al. 1989).

In summary, the data from the rat, dog, and primate studies on hemodiluted or shocked animals indicate that PFC emulsions can effectively replace volume, deliver adequate oxygen, increase oxygen consumption in animals with an oxygen debt, and markedly increase overall survival rate. The data indicate that PFC emulsions can provide a major portion of an animal's oxygen consumption. The ability of PFCs to increase oxygen consumption in shocked animals may also reflect the superior ability of the small, oxygen-laden particles in the emulsion to traverse and oxygenate previously ischemic microcirculation.

Fluosol was tested for efficacy as a blood substitute in clinical trials in Japan and the U.S.A. In the Japanese trials (Ohyanagi et al. 1984; Mitsuno, Ohyanagi et al. 1982; Mitsuno and Ohyanagi 1985), which ran from 1979 to 1982, 270 patients received Fluosol in dosages of 20 to 30 ml/kg. Instead of blood transfusion for replacement of surgical

blood loss or for improvement of acute hemorrhagic anemia. These studies do not report on survival nor were the results of Fluosol-treated patients compared to control groups, leaving the question of efficacy unanswered. The authors determined that Fluosol provided about 17% of the tissue oxygen consumption at an fractional inspired oxygen (FiO2) of 0.5 to 0.6, an amount equal to the plasma contribution. No acute or chronic adverse reactions were observed, except for a transient decline in neutrophils and platelets shortly after infusion. Hemodynamic parameters of patients were either maintained or recovered to normal after treatment with Fluosol. In patients who died of their diseases, there were no organ abnormalities that could be attributed to Fluosol. Although PFC could still be detected in some tissues 7 weeks post-infusion, organs analyzed 7 months after treatment were free of PFC.

In the USA, Fluosol was tested first on a humanitarian protocol. The objectives of this study were to determine clinical safety, hemodynamic, and oxygen transport profiles of Fluosol. In contrast to earlier reports, fully one-third of the patients experienced an acute reaction to infusion of a 0.5-ml test dose of Fluosol which was controlled by treatment with corticosteroids (Tremper et al. 1985). The treated patients had about 3% PFC in their systemic circulation which contributed about 0.7 volume % oxygen to the total oxygen transported, or about 25% of the oxygen consumption of the patient.

A subsequent clinical trial by Gould reported similar results (Gould et al. 1986). The patients in this study, who received up to 40 ml Fluosol/kg, were severely anemic with pretreatment hemoglobin levels of about 3 g/dl. Fluosol made a significant contribution to overall oxygen consumption, providing 28% of the oxygen consumed and demonstrating the ready availability of the oxygen carried by the PFC phase. Despite the significant contribution to oxygen consumption noted in the Gould paper, and in contrast to most of the previously published animal studies, the overall level of oxygen delivery and consumption declined in Fluosol treated patients . Gould concluded that Fluosol failed to make greater contributions to overall oxygen delivery because of the low volume of PFC in the Fluosol formulation and its short intravascular half-life of 24 hours. He speculated that second- generation emulsions with a higher initial concentration of PFC might lead to higher circulating PFC concentrations and better efficacy. He also indicated that a clinical trial where the efficacy of Fluosol was evaluated as a short term replacement until red blood cells became available might have a more favorable outcome. Perhaps the most important aspect of this particular clinical trial was the fact that there were no adverse patient reactions to Fluosol. Gould concluded that Fluosol was safe but not effective, largely because of low PFC content. Despite this outcome, Fluosol continues to be utilized on a humanitarian protocol at certain institutions (Spence et al. 1989).

The fact that Fluosol failed in clinical trials should not obscure the fact that PFCs did make a significant and reproducibly measurable contribution to oxygen consumption in these severely anemic patients, that side reactions to the product were minimal and controllable and that the overall safety of Fluosol was adequate to insure its testing in other clinical indications such as balloon angioplasty and cancer therapy.

6.2 MYOCARDIAL INFARCTION

Numerous studies have demonstrated that mortality and morbidity in acute myocardial infarct (MI) are directly related to the degree of destruction of the myocardial tissue due to ischemia post infarct. The timeframe of effective response to coronary artery occlusion has been shown to be short, on the order of six hours. Thereafter, tissue damage is largely irreversible. Thus, the more rapidly the infarcted myocardium is reoxygenated and reperfused, the more myocardial tissue will be preserved and the final outcome improved for the patient.

PFC emulsions have been proposed as therapeutic agents for preservation of myocardium after an infarct because of their ability to transport oxygen in significant quantities and their small particle size. The small particle size and lowered viscosity of

PFC emulsions suggest that they might flow more readily through the long and thin intercapillary connections that make up the collateral circulation in humans, and thus help to oxygenate the myocardium distal to the occlusion (Faithful et al. 1986). These same properties suggest that PFC particles might reperfuse the edematous "no reflow" vessels resulting from ischemia, bringing in oxygen and removing carbon dioxide in the acidotic tissues functioning under conditions of anaerobic metabolism (Nunn et al. 1983).

Glogar and coworkers assessed the efficacy of PFCs in preservation of ischemic myocardium in dog models (Glogar et al. 1981). After infusion of the PFC emulsion or a comparable amount of LR, the animals breathed 100% oxygen for 15 min. prior to permanent occlusion of the left anterior descending (LAD) artery. After six hours of occlusion, the animals were sacrificed and the area of necrosis (A_N), the area at risk (A_R) and the ratio of A_N/A_R were measured. The area of necrosis (A_N) and the ratio of A_N/A_R were both significantly reduced by about 30% ($p<.01$) in the PFC-treated group compared to the LR and the control groups.

Nunn and coworkers have examined the effect of Fluosol on myocardial salvage in a similar model (Nunn et al. 1983). One hour after ligation of the LAD, Fluosol or 0.9% saline was infused with simultaneous withdrawal of blood to a dose of 30 ml emulsion/kg. The ratio of the A_N/A_R is statistically significantly reduced by about 33% in the Fluosol treatment group.

In studies done in pigs, whose collateral circulation is most similar to humans and lacks the variation found in dogs,
Faithful et al. (1986) evaluated the ability of Fluosol to preserve myocardium during infarct by measuring the change in myocardial oxygen tension polarographically in the most hypoxic region of the ischemic myocardium for five hours post infarct. Oxygen tension of the ischemic myocardium in the PFC-treated animals increased greatly relative to controls.

Minimization of reperfusion damage by Fluosol post MI has been reported by Forman, using both an intracoronary (Kolodgie et al. 1986), and a systemic infusion model (Bajaj et al. 1989). In the earlier intracoronary model, reperfusion with Fluosol after 90 min. of ischemia resulted in a 60% reduction in infarct size and improved ventricular function two weeks post infarct. There was no evidence of increased myocardial oxygen tensions in the Fluosol group compared to control despite ventilation with 100% oxygen during and for three hours after reperfusion. Histologically, there was reduced neutrophil deposition in the microcirculation of the A_R of Fluosol-treated dogs compared to controls. Electron microscopy revealed capillary obstruction involving endothelial cell protrusions and neutrophil and red cell plugging of the obstructed capillaries in control dogs but not in Fluosol treated animals. The authors interpreted this as evidence for improved reflow after the occlusion was eliminated.

In the systemic infusion model (Bajaj et al. 1989), A_N/A_R was significantly reduced after 90 minutes of ischemia in the Fluosol group (24% compared to the LR control) while A_R was similar in both treatment groups at 48-50%. Epicardial blood flow measurements in the central ischemic zone indicate that collateral blood flow variability did not bias the A_N/A_R. Control animals and Fluosol-treated animals had significantly reduced blood flow in the central ischemic zone during occlusion. One hour post reperfusion, the control animals had significantly reduced myocardial blood flow in the epicardial and endocardial ischemic zone compared to Fluosol. There were two- to fourfold increases in neutrophil infiltration into the epicardium, midmyocardium, and endocardium ischemic regions in control dogs compared to Fluosol treated dogs. Non-ischemic regions of the myocardium had similar levels of neutrophils in the LR and Fluosol treated groups. Venous neutrophil counts were lowered within one hour of Fluosol infusion and remained below control counts throughout the reperfusion period. Neutrophils from the Fluosol group exhibited lowered *ex vivo* chemotaxis one hour after reperfusion than control groups. (Bajaj et al. 1989) concluded that the primary mechanism by which Fluosol preserves myocardium is by reducing neutrophil chemotaxis and adhesion in the ischemic tissue during reperfusion.

In summary, PFC emulsions have been shown to reduce relative infarct size, increase myocardial oxygen tension and neutralize inflammatory cells during infarct and after reperfusion. Despite the overwhelming evidence of myocardial preservation during MI in animal studies of PFC emulsions from at least four independent labs, there have no published clinical studies on the use of PFC emulsions to treat acute MI.

6.3 PERCUTANEOUS TRANSLUMINAL CORONARY ANGIOPLASTY (PTCA)

Percutaneous transluminal coronary angioplasty (PTCA) is a procedure that is used to remodel arterial plaque and allow increased coronary artery blood flow. It has gained wide acceptance with over 300,000 angioplasties being performed in the USA annually. In this procedure, a catheter with an inflatable balloon at its tip is threaded into the coronary artery experiencing partial blockade. Once centered in the lesion, the balloon is inflated, compressing the plaque against the vessel wall and increasing the effective diameter of the coronary artery. Current medical practice requires balloon inflation times of 45 seconds or longer to insure optimal results, and there is an interest in increasing the inflation time to determine if (re-occlusion of the artery) rates can be reduced. During the period of balloon inflation, the coronary artery is occluded and part of the left ventricle experiences temporary ischemia. Recent studies have shown that balloon inflation times of 20 seconds are enough to produce ECG changes, induce ventricular wall motion irregularities, and reduce the left ventricular ejection fraction. Such responses are exacerbated in patients with multivessel disease. In order to increase the time of inflation or to reduce risk in patients with multivessel disease, physicians have attempted to perfuse blood through the central lumen of the angioplasty catheter. Blood perfusion was found to have a number of disadvantages including high viscosity, hemolysis, and the necessity of another arterial access point to provide blood for the perfusion.

In the early 1980s, Spears piloted the use of oxygenated Fluosol perfused through the central lumen of the catheter to alleviate symptoms of ischemia during balloon inflation in dogs. The results showed that 8 of 10 dogs perfused with oxygenated Fluosol during prolonged balloon inflation times (>5 min.) had normal ECGs while controls perfused with oxygenated saline had an ECG injury pattern and increased ventricular ectopy (Spears et al. 1983).

Both Spears (Spears et al. 1984) and Anderson (Anderson et al. 1985) issued preliminary reports on the use of Fluosol in humans but the definitive clinical studies were performed by Jaffe and coworkers (Cleman et al. 1986; Jaffe et al. 1988). They used two-dimensional echocardiography to detect the location, extent and evolution of ischemic contractile dysfunction and quantitatively assessed regional wall motion and ejection fraction by computer analysis of the echocardiograms.

In the clinical trial, 42 symptomatic patients with single lesions of 70%-or-greater stenosis of the coronary artery undergoing PTCA were studied. All patients underwent a preliminary inflation without perfusion. Subsequent inflations were done with either oxygenated LR, oxygenated Fluosol or nonoxygenated Fluosol. The data showed that oxygenated Fluosol maintained an ejection fraction identical with the baseline value 45 seconds post balloon inflation, while there was 35% reduction in ejection fraction in the controls. These studies were the basis of the recent FDA approval granted for the use of Fluosol in PTCA on high-risk patients (Anonymous 1990).

6.4 CANCER THERAPY

Solid tumors possess vascular insufficiencies and blood flow irregularities resulting in significant areas of hypoxic cells, frequently amounting to 10-20% of the total viable tumor cell population (Thomlison and Gray 1955). Hypoxia has long been known to protect cells from the cytotoxic effects of radiation and chemotherapy. The surviving hypoxic cell

fraction is generally recognized as capable of reestablishing the tumor and limiting the therapeutic effectiveness of these modalities. There have been numerous efforts to render the hypoxic cell fraction susceptible to radiation and chemotherapy including the development of radiosensitizers such as misonidazole and the use of hyperbaric oxygen (HBO) chambers.

In 1983, Teicher and Rockwell demonstrated that infusion of PFC emulsions in advance of radiation coupled with carbogen (95% oxygen and 5% carbon dioxide) breathing during radiation therapy significantly reduced the surviving fraction of hypoxic cells and the growth rate of implanted rodent tumors (Teicher and Rockwell 1983). Subsequently, Teicher and Rose found that mice bearing tumors from Lewis lung tumor or FSa-II fibrosarcoma treated with Fluosol and breathing carbogen had tumor growth delays of up to 30 days compared to rats receiving either radiation alone, radiation and carbogen alone, or radiation and Fluosol with air breathing (Teicher and Rose 1984). Others have reported similar results (Song et al. 1986; Rockwell 1985; Lustig and McIntosh 1986).

The mechanism of enhancement of radiation therapy appears to be due to increased oxygenation of the tumor. Song et al. (1987) measured the pO_2 of tumors in mice and verified that tumor oxygenation is markedly increased by the combination of Fluosol and carbogen breathing. The results show that tumor oxygenation is increased six-seven-fold in mice breathing carbogen and infused with Fluosol over mice breathing room air and two-fold over mice breathing carbogen without Fluosol. Klubes and coworkers (1987) found that Fluosol does not increase blood flow through solid tumors in the model they studied. Long has documented by X-ray and histological studies that most implanted and spontaneous animal tumors preferentially accumulated PFC emulsion in macrophages associated with the tumors (Long et al. 1978). What, if any, role this phenomenon plays in radiation therapy enhancement is not clear.

Clinical trials of Fluosol in cancer radiation therapy were first reported in 1986 (Rose et al. 1986). These trials were conducted on 15 patients with Stage III/IV head and neck cancer, who were not candidates for surgical therapy. Radiation was fractionated into about. 25 doses of 1.8 Gy over a five-week period. Fluosol was infused at a rate of eight to nine ml/kg on the first day of each week for a total dose of 40-45 ml/kg and patients breathed 100% oxygen before and during all 25 radiation fractions. Of the 15 patients, 10 had primary and nodal clearance with the longest follow-up post treatment being eight months. The authors noted four cases of acute reactions controllable with diphenhydramine and eight of 15 patients exhibited serum enzyme elevations of two to three times normal which returned to normal three months post therapy. Coagulation times, BUN, creatinine, serum albumin and bilirubin were unaffected by the treatment. White blood cell counts and hematocrit were slightly depressed but the changes were typical of normal responses to radiation therapy and could not be attributed to Fluosol. There was some evidence for acceleration of the onset of the mucositis normally caused by the radiation.

Lusting et al. (1989a) have also reported on clinical trials with Fluosol in patients with head and neck cancer. In this study, 37 patients were enrolled and 28 (76%) had complete response (no evidence of primary disease) two months post treatment. The determinant survival, excluding those who died from other causes, was 78% one year post therapy compared to the Radiation Therapy Oncology-Group reported survival rate of 53-62%. Patient side effects were reported to be mild and reversible and of about the same frequency, extent, and duration as observed in the first clinical trial (Rose et al. 1986). While cancer patients are not considered cured until after five years of complete remission, the initial response to Fluosol has been very promising. In addition to the head and neck trials discussed above, clinical trials using Fluosol and radiation therapy for treatment of non-small-cell lung cancer (Lustig et al. 1989b) and brain glioma are underway (Rockwell 1988).

The effects of Fluosol and oxygen breathing were also evaluated in conjunction with chemotherapy. Ohyanagi and coworkers found that animals treated with vincristine or spadicomycin and Fluosol with oxygen breathing had smaller tumor masses compared to animals treated with the chemotherapeutics alone (Ohyanagi et al. 1983). Subsequently, a number of investigators have reported on the potentiating effect of Fluosol on chemotherapy induced tumor growth delay (TGD) (Teicher and Holden 1987). Fluosol with oxygen breathing enhanced the effectiveness of all three major classes of chemotherapeutics: alkylating agents, antibiotics and alkaloids and antimetabolites. Despite the large number of papers on the use of Fluosol with chemotherapeutics, there have been no reports of clinical studies.

6.5 LIQUID BREATHING

Liquid breathing using PFCs was the catalyst for an explosion in research on the use of PFCs as oxygen transport agents. While liquid breathing research was rapidly overshadowed by research on emulsions, the field remained active and now promises to make clinical impact. The list of potential applications of "liquid breathing" can be divided into two major groups: liquid ventilation therapies designed to oxygenate a patient with compromised pulmonary function; and lavage therapies which strive to maintain oxygenation while the lung is purged of obstructive material. Liquid ventilation is being investigated as a therapy for premature infant respiratory distress syndrome (RDS), adult respiratory distress syndrome (ARDS), and "respirator lung"-lungs stressed due to prolonged mechanical ventilation. Lavage therapies under investigation include removal of meconium, fibrotic material in cystic fibrosis, and proteinaceous matter in proteinemia diseases.

After Clark and Gollan's famous demonstration of liquid breathing, Modell and coworkers evaluated the feasibility of
long-term maintenance of dogs and primates via liquid ventilation (Modell et al. 1970b). First they determined that, unlike saline lavages, PFC lavages did not extract surfactant from the lungs of dogs nor did they modify their surface tension properties (Modell et al 1970a). Liquid ventilation of dogs and primates was accomplished with PFC FX-80 perfluorobutyltetrahydrofuran for 30 min. to eight hours. Both species became hypercarbic (arterial CO_2 of 40 to 80) and acidotic (arterial pH of 7.05 to 7.2) as the liquid ventilation progressed but were otherwise well oxygenated. After the PFC had been drained from their lungs, the animals resumed normal air breathing but with significantly depressed arterial oxygen levels of 45-55 mm Hg. Measurement of the PaO_2 over time indicated a return to normal values about 10 days after liquid ventilation. Gross examination of lungs from dogs serially sacrificed from one hour to 10 days after termination of liquid ventilation showed a translucent sheen suggestive of PFC in the dependent alveolar sacs of the one-hour lungs. Three days after liquid ventilation the translucent areas were less extensive and numerous, and after 10 days only a few lobes had a translucent sheen. Microscopically, the lungs from the three-hour post liquid ventilation were hyperemic, contained neutrophil exudate in the bronchioles and had some congested alveolar septa filled with numerous intra-alveolar vacuolated macrophages. Ten days after treatment the lungs were virtually normal microscopically. The authors suggested that the hyperemia and inflammatory reactions were due to the alveolar distention of liquid ventilation or to an irritant effect of the PFC itself or both. They concluded that the reduced PaO_2, which was readily improved by breathing $FiO_2 = 0.4$, was due to the PFC in the alveolar septa forming a diffusion barrier to oxygen transfer. On balance, the data support the conclusion that liquid ventilation does not cause any adverse morphological, biochemical, or histological effects.

The problems of acidosis and hypercarbia were probably related to inadequate removal of metabolic CO_2. To determine if the hypercarbia and acidosis limited the duration of liquid breathing, four dogs were liquid ventilated for eight hours with buffer administered

to prevent acidosis. All four dogs survived, the acidosis was controlled by the buffer, and the hypercarbia leveled off at a 60-80 mm Hg. While all four dogs survived, two who were given higher-pressure ventilations suffered lung tissue damage, suggesting that pressure control during liquid breathing would be necessary. Moskowitz and Schaffer have subsequently developed ventilators for liquid breathing to solve the problems of CO_2 removal and pressure induced tissue damage (Schaffer and Moskowitz 1974).

Modell also studied tissue distribution in dogs and primates several years after liquid breathing. The tissues were examined grossly, microscopically, and chromatographically. The results were unremarkable except for the presence of trace amounts (a few milligrams per 100 g of tissue) of PFC in the lungs, liver, and fatty tissue (Modell et al. 1976).

In summary, liquid ventilation studies with adult dogs and primates resulted in adequate oxygenation during liquid breathing accompanied by acidosis and hypercarbia. After liquid ventilation, PaO_2 levels were depressed for three to 10 days, probably due to PFC in the alveolar sacs. After 10 days and for periods thereafter up to three-years post liquid ventilation, lungs were normal grossly, microscopically, and biochemically, with the exception of traces of PFC in various tissues

In studies with immature and premature animals, the results were markedly different. Rufer and Spitzer studied the use of liquid ventilation with PFC in immature pigs (Rufer and Spitzer 1974). In pigs of 95-days gestation, air ventilation was difficult due to atelectasis, and 75% of the animals died within 15 min. At 100 days gestation, air ventilation was maintained for 90 min. and at 110 days gestation (full term), air ventilation was easily achieved and survival was high. Rufer and Spitzer found that liquid ventilation of the 95-day immature mini-pig could be sustained for over three hours and that the animals did not become hypercarbic. A more exciting finding was that compliance in the immature lung after liquid ventilation was improved almost to that of a gestationally mature mini-pig in spite of the fact that lung lavage of the 95-day gestational animals failed to recover any surfactant. It appeared that PFC assumed the role of surfactant in the immature lung, decreased the surface tension of the lung, and restored compliance towards normal during subsequent air breathing. The results suggest that periods of liquid breathing need not be terribly long to have lasting benefit. Schaffer and coworkers found that premature sheep were readily ventilated with PFCs and that peak intratracheal pressures were significantly reduced after liquid ventilation (Schaffer et al. 1976). Schaffer's work also confirmed that hypercarbia and acidosis were not as extreme in the immature lung undergoing liquid ventilation. In a subsequent paper, Schaffer and coworkers found that pre-term lambs could be liquid ventilated for three hours and that their gas exchange and lung compliance were similar to mature lambs undergoing gaseous ventilation. They believe that this result extends the viability of the pre-term lamb to the limit of the pulmonary capillary development rather than that of the pulmonary surfactant system (Schaffer et al. 1983).

Schaffer and coworkers also found that cardiac output is decreased (Lowe et al. 1979) and pulmonary vascular resistance is increased during liquid breathing in pre-term lambs (Lowe and Schaffer 1986). Modell and coworkers on the other hand, found no change in cardiac output in adult dogs (Modell et al. 1970b). The differences in these reports may be due to differential response in premature animals compared to adults, species differences, or methodological differences. If cardiac output decrease and PVR increase are confirmed during liquid breathing, it may have consequences for long-term maintenance via liquid breathing. However, effects would have no impact on the short-term usage required for the PFC to serve as a temporary surfactant and open the alveoli.

Waldrop (1989) conducted a clinical trial on a 24 to 28-week-old human infant, with 15 min. of liquid breathing. Although the baby died, the lungs functioned for 19 hours after the liquid ventilation ceased. Further trials are planned in premature infants.

7. Second Generation Products

At least five companies are in the development stage with second generation products. HemaGen/PFC (San Francisco, CA.), Green Cross Co. (Osaka, Japan), Alliance Pharmaceutical (San Diego, CA.), Dupont (Wilmington, De.) and Affinity Biotech (Linwood, PA.). HemaGen has developed an emulsion based on the use of triglyceride oils to improve surfactant interaction with the PFC, resulting in emulsions with improved stability at PFC contents as high as 70 vol%. Invented by R.F. Shaw, a physician and medical entrepreneur and L.C. Clark, the original PFC pioneer, these high-PFC emulsions can be sterilized at 121°C for as long as 30 min. without degradation or cracking (Shaw and Clark 1987). The mean particle size after sterilization is 0.2 μm by laser light scattering, and the emulsions can be stored for over a year at 4 and 25°C. Particle size does not increase during storage. The viscosity of the emulsion is 7 cP at 37°C, and it does not impair flow through the microcirculation. Animal safety is several times greater than Fluosol DA-20 on a ml PFC/kg basis.

Green Cross Co. (Osaka, Japan) recently published information on a second-generation emulsion based on lecithin and perfluoro-N-methyldecahydroisoquinoline (Tsuda and Yokoyama 1989). The emulsion is reported to be only a 10 vol% emulsion and is stable at "cold room temperature." Perfluoro-N-methyldecahydroiso- quinoline reportedly has a short tissue half-life.

Alliance Pharmaceutical (San Diego, CA.) has developed an emulsion based on high perfluorooctyl bromide content and using high concentrations of lecithin as the surfactant (Burgan et al. 1987). The formulation was published as a 6% egg yolk lecithin and 100 (wt/vol)% perfluorooctyl bromide (about 52 vol%) perhaps including coadditives such as tocopherol. The LD_{50} in mice was reported to be 45 g emulsion/kg or about 32 ml emulsion/kg. Perfluorooctyl bromide was widely investigated as an oxygen transport agent in the 1970s by other investigators including Clark and Yokoyama but was not developed. Long, who had been investigating the utility of bromoperfluorochemicals as radiographic contrast agents for many years, developed the emulsion which is now being evaluated in clinical trials. Total exchanges using the same formulation have been reported. Mean particle size is reported to be 0.2 μm, and the emulsion is reported to be shelf stable at room temperature (Long et al. 1987). Hemodynamic responses in dogs were negligible with this emulsion compared to the well-known hemodynamic collapse in dogs when given Fluosol DA-20® (Mattrey et al. 1989).

Dupont has developed an emulsion called Therox® using lecithin and containing 40 volume% 1, 2-bis(perfluorobutyl)ethylene. The emulsion is shelf stable at 4°C for over one year, has a PSD mean of about 0.25μ, has a viscosity compatible with whole blood and is well tolerated in animals. 1, 2-bis(perfluorobutyl)ethylene is expired from the liver and spleen with a half-life of 3-18 days. The clinical status of this product is unknown.

These second-generation products have solved two of the more serious problems with Fluosol DA-20: the acute complement activation caused by pluronic F-68 and the necessity to be stored frozen.

8. Conclusion

PFCs have come a long way since Clark's first experiment and the ensuing euphoric period when too much was expected and too little was known. Over the intervening years, the complexities of formulating, manufacturing, testing, and utilizing PFC emulsions have been unraveled and the first PFC product, Fluosol DA-20, is readily available in the USA. Unfortunately, Fluosol DA-20 proves inadequate for the goals set for it. Nonetheless, it

served to define the next level of hurdles that the second-generation of oxygen transport agents will have to clear to achieve widespread use. Fluosol DA-20 was also responsible for the development of a body of knowledge based on the preclinical and clinical efficacy testing of PFC based products. Medical understanding of PFCs and of their use have been brought into focus by the Fluosol DA-20 experience. The finding that pluronic F-68 was responsible for the undesired acute reactions has led to its removal from second- generation products. The benign and reversible side effects attributable to PFCs now are fairly well understood and are not a threat to organ function at therapeutic doses. Several new formulations have been developed that will expand the uses of PFC emulsions into more applications. Research on third- generation products that will persist significantly longer in the bloodstream is underway, and the first new classes of safe intravenous surfactants have been identified. After a somewhat- long induction period, the use of PFC oxygen transport agents in medicine is poised to enter an exponential growth phase.

REFERENCES

Anonymous (1990) 'Alpha Therapeutic's Fluosol oxygen transport fluid approved for use in angioplasty; launch will be initiated at cardiologist conference in March', FDC Reports 52, 8.

Bajaj, A.K., Cobb, M.A., Virmani, R., et al. (1989) 'Limitation of myocardial reperfusion injury by intravenous perfluorochemicals', Circulation 79, 645-656.

Blumberg, N., Agaral, M. and Chuang, C. (1985) 'A possible association between survival time and transfusion in patients with cervical cancer', Blood 66 (suppl. 1), 274a.

Burgan, A.R., Long, D.M., Mattrey, R.F., et al. (1987) 'Results of pharmacokinetic and toxicologic studies with PFOB emulsions', Biomaterials, Artificial Cells, and Artificial Organs 15, 367.

Clark, L.C. and Gollan, F. (1966) 'Survival of mammals breathing organic liquids equilibrated with oxygen at atmospheric pressure', Science 152, 1755-1756.

Clark, L.C., Wesseler, E., Miller, M. and Kaplan, S. (1974) 'Ring versus straight chain perfluorocarbon emulsions for perfusion media', Microvascular Research, 8, 320-340.

Cleman, M., Jaffe, C.C., and Wohlgelernter, D. (1986) 'Prevention of ischemia during percutaneous transluminal coronary angioplasty by trans-catheter infusion of oxygenated Fluosol DA-20%', Circulation 74, 555-562.

Elliott, L.A., Ledgerwood, A.M., Lucas, C.E., et al. (1989) 'Role of Fluosol-DA 20% in prehospital resuscitation', Critical Care Med. 17, 166-172.

Faithful, N.S., Erdmann, W., Fennema, M. and Kok, A. (1986) 'Effects of haemodilution with fluorocarbons or dextran on oxygen tensions in the acutely ischemic myocardium', Brit. J. Anesth. 58, 1031-1040.

Geyer, R.P., Monroe, R.C., and Taylor, K. (1968) 'Survival of rats totally perfused with a fluorocarbon-detergent preparation', in Organ Perfusion and Preservation (Norman, J., ed.), pp. 85-97, Appleton Century and Crofts, New York.

Geyer, R.P.,Taylor, K., Duffett, E., et al. (1973) 'Successful complete replacement of the blood of living rats with artificial substitutes', Fed. Proc. 32, 927.

Gjaldbaek, J., and Hildebrand, J.H. (1949) 'The solubility of nitrogen in carbon disulfide, benzene, normal and cyclo-hexane and in three fluorocarbons', J. Am. Chem. Soc. 71, 3147.

Glogar, D.H., Kloner, R.A., Muller, J., et al. (1981) 'Fluorocarbons reduce myocardial ischemic damage after coronary occlusion', Science 211, 1439-1441.

Gould, S.A, Rosen, A.L, Sehgal, L.R., et al. (1986) 'Transfusion trigger literature', New England J. Med. 314, 1653-1656.

Haljamae, H., and Rosenberg, P.H. (1988) 'Perioperative transfusion practice-An update', Acta Anaesthesiol. Scand. 32 (suppl. 89), 1-3.

Hamza, M.A., Serratrice, G., Stebe, M-J., Delpuech, J-J. (1981) 'Solute-solvent interactions in perfluorocarbon solutions of oxygen. An NMR study', J. Am. Chem. Soc. 103, 3733-3738.

Huestis, D.W., Bove, J.R., and Busch, S. (1981) Practical Blood Transfusion, Little Brown and Company, Boston.

Jaffe, C.C., Wohlgelernter, D., and Cabin, H., et al. (1988) 'Preservation of left ventricular ejection fraction during percutaneous transluminal coronary angioplasty by distal transcatheter coronary perfusion of oxygenated Fluosol DA 20%', Am. Heart J. 6, 1156-1164.

Klubes, P.S., Hiraga, S., Richard, L.C., Owens, E.S. and Blasberg, R.G. (1987) 'Attempts to increase intratumoral blood flow in the rat solid Walker 256 tumor by the use of perfluorocarbon emulsion Fluosol-DA', Eur. J. Cancer Clin. 23, 1859-1867.

Kolodgie, F.D., Dawson, A.K., Roden, D.M., et al. (1986) 'Effect of Fluosol-DA on infarct morphology and vulnerability to ventricular arrhythmia', Am. Heart J. 112, 1192-1201.

Liu, M.S., Rosen, A., and Long, D.M. (1973) 'Biological disposition of perfluorooctylbromide-A new contrast agent', Fed. Proc. 32, Abstr. No. 3982, 927.

Long, D.M., Multer, F.K., Greenburg, A.G., et al. (1978) 'Tumor imaging with X-rays using macrophage uptake of radiopaque fluorocarbon emulsions', Surgery 84, 104-112.

Long, D.C., Fallano, R., Reiss, J.G., et al. (1987) ' Preparation and applications of highly concentrated PFOB emulsions', Biomaterials, Artificial Cells, and Artificial Organs 15, 417.

Lowe, C.A., and Schaffer, T.H. (1986) 'Pulmonary vascular resistance in the fluorocarbon-filled lung', J. Appl. Physiol. 60, 154-159.

Lowe, C., Tuma, R.F., Sevieri, E.M. and Schaffer, T.H. (1979) 'Liquid ventilation: cardiovascular adjustments with secondary hyperlactatemia and acidosis.', J. Appl. Physiol.:Respirat. Environ. Exercise Physiol. 47, 1051-1057.

Lustig, R.A., and McIntosh, N. (1986) 'Fluosol-DA in radiation therapy', Prog. Clin. and Biol. Res. 211, 29-38.

Lustig, R., McIntosh-Lowe, N., Rose, C., et al. (1989a) 'Phase I/II study of Fluosol-DA and 100% oxygen as an adjuvant to radiation in the treatment of advanced squamous cell tumors of the head and neck.', Int. J. Radiation Oncology Biol., and Phys. 16, 1587-1593.

Lustig R.A., Lowe, N., Prosnitz, L., et al. (1989b) 'Phase I/II study of Fluosol and 100% oxygen breathing as an adjuvant to radiation in the treatment of unresectabel non small cell carcinoma of the lung', Int. J. Radiation Oncology, Bio., and Phys 17, 202.

Makowski, H. (1978) 'The properties of Fluosol-DA infusion in the treatment of hemorrhagic shock', in Proc. 4th International Symposium on Perfluorochemical Blood Substitutes, Oct. 21-22, 1978, Kyoto Japan 439-448, Excerpta Medica, Amsterdam.

Mattrey, R.F., Hilpert, P.L., Long, C.D. et al. (1989) 'Hemodynamic effects of intravenous lecithin-based perfluorocarbon emulsions in dogs', Critical Care Medicine 17, 652-656.

Mitsuno, T., Ohyanagi, H., and Naito, R. (1982) 'Clinical studies of a perfluorochemical whole blood substitute (Fluosol-DA): Summary of 186 cases', Ann. Surg. 195, 60-69.

Mitsuno, T., and Ohyanagi, H. (1985) 'Present status of clinical studies of Fluosol-DA (20%) in Japan in Tremper, K. (ed), International Anesthisiology Clinics. Perfluorochemical Oxygen Transport, Little, Brown and Company Publishers, Boston pp. 169-184.

Modell, J.H., Gollan, F., Giammona, S.T. and Parker, D. (1970a) 'Effect of fluorocarbon liquid on surface tension properties of pulmonary surfactant', Chest 57, 263-265

Modell, J.H., Newby, E.J. and Ruiz, B.C. (1970b) 'Long-term survival of dogs after breathing oxygenated fluorocarbon liquid', Fed. Proc. 29, 1731-1736.

Modell, J.H., Hood, C.I., Kuck, E.J. and Ruiz, B.C. (1971) 'Oxygenation by ventilation with fluorocarbon liquid (FX-80)', Fed. Proc. 34, 312-320.

Modell, J.H., Calderwood, H.W., Ruiz, B.C., Tham, M.K., and Hood, C.I. (1976) 'Liquid ventilation of primates', Chest 69, 79-81.

Moore, R.E., and Clark, L.C. (1985) 'Chemistry of fluorocarbons in biomedical use', in Tremper, K. (ed), International Anesthisiology Clinics. Perfluorochemical Oxygen Transport, Little Brown and Company Publishers, Boston pp. 11-24.

Nunn, G.R., Dance, G., Peters, J., and Cohn, L.H. (1983) 'Effect of fluorocarbon exchange transfusion on myocardial infarction size in dogs', Am. J. Cardiol. 52, 203-205.

Ohyanagi, H., and Mitsuno, T. (1975) 'Biophysiological effects of perfluorochemicals as artificial blood', in Proc. of the Post Congress Symposium, Xth International Congress of Nutrition on Perfluorochemical Blood, Kyoto, Japan, pp.21-54.

Ohyanagi, H., Sekita, M., Yokoyama, K., et al. (1978) 'Studies on perfluorochemical emulsion (Fluosol DA) as artificial blood in exchange transfusion and emergency use in monkeys', in Proc. of the 4th International Symposium on Perfluorochemical Blood Substitutes, Oct. 21-22, 1978, Kyoto Japan, pp.373-389. Excerpta Medica, Amsterdam

Ohyanagi, H., Nishijima, M., Usami, M., et al. (1983) 'Experimental studies on the possible combined chemotherapy to neoplasms with Fluosol-DA infusion', Prog. Clin. Biol. Res. 211, 315-320.

Ohyanagi, H., Nakaya, S., Okumura, S., and Saitoh, Y. (1984) 'Surgical use of Fluosol-DA in Jehovah's Witness patients', Artificial Organs 8, 110-118.

Okada, K., Kosugi, I., Kawashima, Y. et al. (1975) 'Effect of Fluosol-DC on tissue pO_2 and pCO_2 in treatment of hemorrhagic hypotension', in Proc. of the Post Congress Symposium, Xth International Congress of Nutrition on Perfluorochemical Blood, Kyoto, Japan, pp.215-224.

Reiss, J.G. and LeBlanc, M. (1978) 'Perfluoro compounds as blood substitutes' Angew. Chem. Int. Ed. Engl., 17, 621-634.

Reiss, J.G. (1984) 'Reassessment of Criteria for the selection of perfluorochemicals for second-generation blood substitutes: Aanalysis of structure/property relationships', Artif. Organs, 8, 44-56.

Rockwell, S. (1985) 'Use of perfluorochemical emulsion to improve oxygenation in a solid tumor', Int. J. Radiation Oncology, Biol. and Phys. 11, pp.97-103.

Rockwell, S. (1988) Personal Communication, Yale Medical Center, Hunter Radiation Center, New Haven, CT.

Rose, C. M., Lustig, R., McIntosh, N., and Teicher, B. (1986) 'A clinical trial of Fluosol DA 20% in advanced squamous cell carcinoma of the head and neck', Int. J. Radiation Oncology, Biol. and Phys. 12, 1325-1327.

Rosen, A.L., Sehgal, L.R., Gould, S.S. Sehgal, H.L., Moss, G.S. (1985) 'Transport of oxygen by perfluorochemical emulsions', in Tremper, K. (ed), International Anesthisiology Clinics. Perfluorochemical Oxygen Transport, Little Brown and Company Publishers, Boston pp. 95-103.

Rosenblum, W.I., Moncure, C.W. and Behm, F.G. (1985) 'Some long-term effects of exchange transfusion with fluorocarbon emulsions in macaque monkeys', Arch. Pathol. Lab Med. 109, 340-344.

Rufer, R., and Spitzer, H. L. (1974) 'Liquid ventilation in the respiratory distress syndrome', Chest 66(suppl.) 29S-30S.

Schaffer, T.H., and Moskowitz, G.D. (1974) 'Demand-controlled liquid ventilation of the lungs', J. Applied Physiology 36, 208-213.

Schaffer, T.H., Rubenstein, D., Moskowitz, G.D. and Delivoria-Papadopoulos, M. (1976) 'Gaseous exchange and acid-base balance in premature lambs during liquid ventilation since birth', Pediat. Res. 10, 227-231.

Schaffer, T.H., Tran, N., Bhutani, V. K., and Sivieri, E.M. (1983) 'Cardiopulmonary function in very preterm lambs during liquid ventilation', Pediat. Res. 17, 680-684.

Shaw, R.F. and Clark, L.C. (1987) 'Stable emulsions of highly fluorinated organic compounds' European patent application no. 0 231 091.

Sloviter, H., and Kamimoto, T. (1967) 'Erythrocyte substitute for perfusion of brain', Nature 216, 458.

Song, C.W., Zhang, W.L., Pence, D.M., et al. (1986)' Increased radiosensitivity of tumors by perfluorochemicals and carbogen', Int. J. Radiation Oncology, Biol. and Phys. 12, 934-936.

Song, C.W., Lee, I., Hasegawa, T., et al. (1987) 'Increase in pO2 and radiosensitivity of tumors by Fluosol-DA (20%) and carbogen', Cancer Res. 47, 442-446.

Spears, J.R., Serur, J., Baim, D.S. et al. (1983) 'Myocardial protection with Fluosol-DA during prolonged coronary balloon occlusion in the dog', Circulation 68 (suppl. III), 317.

Spence, R.K., McCoy, S., Costabile, J., et al. (1989) 'Fluosol DA-20% in treatment of severe anemia: A randomized, controlled study of 47 patients', Critical Care Med. 17, S144.

Suyama, T., Matsumoto, T., Watanabe, M., Hamano, T., and Naito , R. (1975) 'Exchange transfusion with perfluorodecalin emulsion (Fluosol-DC) in severely hemodiluted dogs', in Proc. of the Post Congress Symposium, Xth International Congress of Nutrition on Perfluorochemical Blood, Kyoto, Japan, pp. 225-235.

Teicher, B.A., and Holden, S.A. (1987) 'Survey of the effect of adding Fluosol-DA 20% O2 to treatment with various chemotherapeutic agents', Cancer Treatment Reports 71,

Teicher, B., and Rockwell, S. (1983) 'Increased efficacy of radiotherapy in mice treated with perfluorochemical emulsions plus oxygen', Amer. Assoc. of Cancer Res. Abst. 25-28, May 1983.

Teicher, B.A., and Rose, C.M. (1984) 'Perfluorochemical emulsions can increase tumor radiosensitivity', Science 223, 934-936.

Thomlinson, R.H., and Gray, L.H. (1955) 'The histological structure of some human lung cancers and the possible implications for radiotherapy', Brit. J. Cancer 9, 539-549.

Tremper, K.K., Levine, E.M. and Waxman, K. (1985) 'Clinical Experience with Fluosol-DA (20%) in the United States', in Perfluorochemical Oxygen Transport (Tremper, K., ed.), Little Brown and Company, Boston, pp 185-197.

Tsuda, Y. and Yokoyama, K. (1989) 'Perfluorochemical emulsions: The industrial view', Abstract no. 698 International Chemical Congress of Pacific Basin, Dec. 17-22, 1989, Honolulu, Hawaii.

Waldrop, M.M. (1989) 'The (liquid) breath of life', Science 245, 1043-1045.

Yokoyama, K., Yamanouchi, K., and Watanahi, M., et al. (1975) 'Preparation of perfluorodecalin emulsion, an approach to the red cells substitute', Fed. Proc. 34, 1478-1483.

Yokoyama, K., Suyama, T. and Naito, R. (1982) 'Selection of 53 PFC substances for better stability', Proc. Int. Sym., San Francisco, Ca. Sept. 29-Oct. 1, pp 189-196.