Blood substitutes: evolution and future applications

MITCHELL G. SCOTT, DENNIS F. KUCIK, LAWRENCE T. GOODNOUGH, and TERRI G. MONK

The development of oxygen-carrying blood substitutes has progressed significantly in the last decade with phase I and phase II clinical trials of both hemoglobin-based and perfluorocarbon-based oxygen carriers nearing completion. As these products approach clinical use it is important for the laboratory medicine community to be aware of their effects on routine laboratory testing and the settings in which they might be used. Here we review the forces driving the development of oxygen-carrying blood substitutes, the clinical settings in which they might be used, the major categories of oxygen carriers in clinical trials, and the challenges faced by these products as they approach clinical use.

The desire for a safe and effective substitute for blood dates back at least as far as the 17th century [1]. Blood substitutes have long been sought for replacement of “bad humor,” treatment of chronic and acute anemias, and rapid replacement of blood lost after trauma [1–4]. The latter indication has understandably stimulated a great deal of interest from the military services [5]. During the last decade prospects for a safe and effective blood substitute have appeared on the horizon [1–4] with products based on soluble hemoglobin (Hb) or emulsions of perfluorocarbons (PFC) currently in clinical trials [6, 7].

It is important for the laboratory medicine community to understand the forces driving blood substitute development, as well as the two main types of blood substitutes under current clinical investigation, as they begin to appear in samples for clinical laboratory testing. As will become apparent in some of the other articles in this issue, these substances can affect the validity of many clinical laboratory tests. It is also important to note that blood substitutes are not considered to be blood products, but are simply oxygen-carrying volume-replacement solutions. They do not contain cells, antibodies, coagulation factors, or any of the other myriad components of blood itself and are more properly referred to as oxygen carriers. Efforts to develop artificial cells [8] or to culture red blood cells (RBCs) from stem cells [9] will not be discussed here.

Why Blood Substitutes?

Some of the problems that were historical driving forces for blood substitutes have been solved by modern medicine. For example, removal of “bad humor” is today accomplished by apheresis, whereas many chronic anemias are treated with iron, B[12]superoxide dismutase.

The use of RBC transfusions to restore the oxygen-carrying capacity of blood in acute anemias is well established, but both real and perceived problems with the blood supply (Table 1) have resulted in efforts to minimize RBC transfusion [13]. These same concerns also heighten interest in a safe approach to restore volume and O2-carrying capacity without allogeneic transfusions.

Probably the most significant concern regarding the blood supply in the US giving impetus to the development of oxygen carriers is the anticipated increased demand for blood, together with a decreasing blood supply. In 1992, 15 million units of blood were collected in the US, with ~60% of all units used in surgical procedures [3, 14–17]. Furthermore, >50% of transfusions occur in patients over the age of 65, a segment of the US population that will double in the next 30 years [3, 14–17]. Thus, as the “baby boomer” population ages and requires more

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2 Nonstandard abbreviations: Hb, hemoglobin; PFC, perfluorocarbon; RBC, red blood cell; ANH, acute normovolemic hemodilution; HBOC, hemoglobin-based oxygen carrier; PTCA, percutaneous transluminal coronary angioplasty; RES, reticuloendothelial system; DPG, diphosphoglycerate; and SOD, superoxide dismutase.
Table 1. Reasons to minimize allogeneic RBC transfusions.

<table>
<thead>
<tr>
<th>Category</th>
<th>Reason</th>
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<tr>
<td>Increasing demand</td>
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<tr>
<td>Decreasing supply</td>
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<td>Safety</td>
<td>Infectious disease transmission</td>
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<td></td>
<td>Transfusion reactions</td>
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<td>Immunosuppression</td>
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<td>Cost</td>
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blood, the prime donor age population will shrink in proportion to the largest population utilizing allogeneic blood. Taken together, a shortage of 4 million units/year has been projected by the year 2030 [4, 16].

Another factor favoring the development of oxygen carriers is the well-known concern about safety of the blood supply and in particular, the public attention focused on infectious disease transmission. While such concerns obviously merit attention, the remarkable efforts of the blood banking and laboratory medicine communities to develop and implement sensitive screening tests have greatly minimized these risks in most developed nations [18]. For instance, in the US the risk of acquiring an infectious disease from allogeneic blood ranges from 1:60 000 for hepatitis B to 1:500 000 for HIV, with transmission rates for other viruses such as hepatitis C and human T-cell leukemia virus intermediate between these [19]. The recent introduction of HIV antigen testing will likely further decrease the risk of HIV transmission. The risk of acquiring bacterial infection from RBC transfusion is even lower than that for HIV [20]. Other safety issues regarding the blood supply relate to severe transfusion reactions. These are most often the result of clerical errors and estimated to occur at a frequency of ~1:12–30 000 [20]. Finally, allogeneic blood has been shown to be immunosuppressive, which may lead to increased risks for nosocomial or other infections after transfusion [21].

Despite the increased public awareness of issues relating to safety of the blood supply, the overall risk of death after allogeneic transfusion is estimated at 1:500 000–1 000 000 [17, 20]. When compared with the annualized 1:50 000 risk of death in an automobile accident in the US [22], the remarkable safety of the blood supply becomes readily apparent. Thus, despite blood safety being a driving force for the development of blood substitutes, the overall safety of the blood supply in the US actually sets rather lofty safety targets for the new oxygen carriers. In contrast to the safety of the blood in the US, the risks of infectious disease transmission, particularly HIV, in developing countries is extraordinarily high [23]. Therefore, on a worldwide basis, safety of the blood supply must be considered a primary reason for blood substitute development.

Blood donation, screening, storage, and administration are estimated to cost our institution ~$150.00 a unit, with laboratory screening and cross-matching contributing ~$25 to this cost. Developers of oxygen carriers need to consider these as maximum costs for institutions that might use these products. Another limitation of the current blood supply is the limited shelf life (~6 weeks at 4°C) and costs associated with storage. An obvious goal for developers of these substances, therefore, is a low-cost product with a long shelf life.

While blood substitute development is being encouraged by the above concerns, the blood banking community also continues to address these issues. A 1987 NIH consensus lowered the transfusion “trigger” to 80 g/L Hb [24]. The use of autologous blood for elective surgeries [25], particularly in combination with recombinant erythropoietin [10], is another successful strategy for minimizing the use of allogeneic blood. Perioperative acute normovolemic hemodilution (ANH) is a safe and effective technique for conserving blood in certain elective surgery settings [26]. In this procedure, several units of blood are taken from a patient immediately before surgery to decrease Hb concentrations to 70–90 g/L. Normovolemia is maintained by volume replacement with crystalloids or colloid solutions. Blood lost during the surgical procedure thus contains fewer RBCs. The patient’s own blood is returned when a transfusion trigger is reached or at the completion of surgical blood loss. These blood conservation efforts are beginning to have an impact on utilization. Since 1989 the use of allogeneic blood is down ~4% in the US, and autologous donations account for 5% of transfusions in the US [3]. In some centers allogeneic blood use has decreased 30–50% in elective surgeries [1].

Although the same issues promote the development of both blood conservation strategies and oxygen carriers, the two approaches are not exclusive. It is unlikely that either conservation strategies or blood substitutes alone will eliminate the projected shortages of blood. Furthermore, blood substitutes will not be applicable in many clinical settings such as the anemia of chronic renal failure, severe blood loss after trauma, leukemic anemias, or chemotherapy-induced anemia. Indeed, most projections suggest that blood substitutes will decrease the use of allogeneic transfusions by only 10–20% [4, 27, 28].

Projected uses for these oxygen carriers are primarily acute settings where long-term needs for volume and oxygen-carrying supplementation are not anticipated (Table 2). Oxygen carriers might logically be used in conjunction with ANH. Rather than replacing the volume lost before surgery with a crystalloid or colloid solution, an oxygen-carrying blood substitute would be used. This might allow a greater amount of the patient’s own blood to be drawn and set aside for later use, or delay the return of the patient’s own blood, thus minimizing the necessity of allogeneic transfusion. Similarly, use of a blood substitute during surgery where blood loss is greater than anticipated might postpone or negate the need for transfusion. In cardiothoracic surgery the use of oxygen carriers for “priming” the bypass pump and for volume replacement is also anticipated to reduce transfusion requirements [29]. Another potential use is replacement of...
acute blood loss after trauma. Replacement of fluid and oxygen-carrying capacity on the battlefield, in an ambulance, or in an emergency department with a blood substitute might speed stabilization of the patient, increase survival, and (or) decrease transfusion requirements without the storage and cross-matching requirements of whole blood. Thus, major blood-loss surgical procedures, cardiothoracic surgeries, and trauma are the major settings in which phases I and II safety and dose-escalation trials are being conducted with several Hb- and PFC-based oxygen carriers.

The only use for which an oxygen-carrying blood substitute is currently FDA-approved may not be as readily apparent as those previously mentioned. Both Hb-based oxygen carrier (HBOC) molecules and PFC emulsion particles (<0.1 μm in diameter) are orders of magnitude smaller than RBCs (~7 μm in diameter). As such, they have much better rheologic properties and are able to diffuse into and deliver O₂ to poorly vascularized hypoxic tissues. The PFC emulsion Fluosol (Green Cross Co.) has FDA approval for use in percutaneous transluminal coronary angioplasty (PTCA) [30]. Other uses that could potentially take advantage of the ability of oxygen carriers to diffuse into the microcirculation include O₂ delivery to poorly vascularized radiosensitive tumors [31, 32] and ischemic crises in sickle cell disease [33].

### Types of Blood Substitutes

**HB SOLUTIONS**

Interest in the use of soluble Hb as a blood substitute first appeared in the literature in 1934 when totally exsanguinated sheep were transfused with a solution of bovine Hb [34]. Attempts at human transfusion with Hb solutions occurred in the late 1940s but met with little success [35]. Patients had anaphylactic symptoms, severe renal toxicity, and hypertension. These symptoms were harbingers of some of the challenges faced by HBOC blood-substitute development today. The challenges can be categorized into availability, immunologic properties, short half-life, excessive affinity for O₂, and vasoactive properties.

**Availability.** Whereas one of the primary reasons for developing oxygen carriers is ready availability to ease the projected shortage in the blood supply, some approaches for HBOCs face similar supply challenges. It is estimated that 70,000 kg of Hb would be required to replace 20% of RBC transfusions in the US [27]. This presents a considerable challenge to human HBOC products. Producers of human HBOCs utilize the Hb from expired RBC products (Table 3) and the projected blood supply shortage might be expected to adversely affect this approach. Production of human Hb by recombinant DNA technology is another approach taken by some producers but whether this technology is capable of producing these massive quantities is unclear [27]. One interesting twist on recombinant technology is the engineering of transgenic pigs that are reported to stably produce up to 50% human Hb [36]. This source is estimated to require ~100,000 transgenic pigs [27], an expensive and lengthy proposition. In contrast, HBOC manufacturers that base their products on bovine Hb are unlikely to face a supply problem. However, the use of nonhuman Hb raises other issues, as discussed below.

**Immunologic properties.** In early attempts to use Hb solutions as oxygen carriers, anaphylactoid side effects were common [35]. These were mainly due to the phospholipid

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### Table 2. Projected clinical uses for blood substitutes.

<table>
<thead>
<tr>
<th>Elective surgery</th>
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<tr>
<td>Preoperative ANH</td>
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<tr>
<td>Perioperative volume replacement</td>
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<td>Cardiovascular surgery</td>
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<tr>
<td>Pump priming</td>
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<tr>
<td>Volume replacement</td>
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<tr>
<td>Trauma</td>
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<tr>
<td>Volume replacement/stabilization</td>
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<tr>
<td>Perfusion of ischemic tissue</td>
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<td>In thrombolytic therapy</td>
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<td>PTCA</td>
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<tr>
<td>Sickle cell crisis</td>
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<tr>
<td>Stroke</td>
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<td>Peripheral vascular disease</td>
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<td>Oxygenation of solid tumors</td>
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<tr>
<td>Radiotherapy</td>
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<td>Chemotherapy</td>
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### Table 3. HBOCs.

<table>
<thead>
<tr>
<th>Hb Source</th>
<th>Modification</th>
<th>Company</th>
</tr>
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<tbody>
<tr>
<td>Bovine</td>
<td>Glutaraldehyde polymerization</td>
<td>Biopure</td>
</tr>
<tr>
<td>Bovine</td>
<td>Polyethylene glycol polymerization</td>
<td>Enzon</td>
</tr>
<tr>
<td>Bovine</td>
<td>O-Raffinose cross-linked</td>
<td>Hemosol</td>
</tr>
<tr>
<td>Expired human RBC</td>
<td>Dibromosalicylate bisfumarate</td>
<td>Baxter</td>
</tr>
<tr>
<td></td>
<td>Cross-linked α chains</td>
<td></td>
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<tr>
<td>Expired human RBC</td>
<td>Glutaraldehyde polymerization</td>
<td>Northfield Labs</td>
</tr>
<tr>
<td></td>
<td>Pyridoxylated 2,3-DPG site</td>
<td></td>
</tr>
<tr>
<td>Recombinant human</td>
<td>Covalent α chain dimers</td>
<td>Somatogen</td>
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<tr>
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<td>Hb Presbyterian</td>
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content of residual RBC stroma that nonspecifically activated the complement cascade. Today, ultrafiltration and purification techniques result in stroma-free HBOC solutions [37]. Another immunologic challenge facing the nonhuman HBOC products is the possibility of specific immune responses to the foreign Hb molecule. These concerns have been lessened by studies demonstrating that Hbs in general are very poor immunogens [38, 39] and that the intravenous route of administration is a poor way to generate an immune response [40]. Immune responses to foreign Hb have apparently not been a problem in phase I and II trials, but whether a patient has ever received multiple doses of these products is unknown. Finally, there are conflicting animal studies that report that soluble Hbs are immunosuppressive and increase the risk of acquiring infections [41, 42]. Several studies suggest that the Hb molecule suppresses macrophage function, thereby increasing the risk of peritonitis and other infections [41, 43].

Short half-life. Hb consists of four noncovalently associated chains (two α and two β), each with one heme molecule for O2 binding. Outside the RBC milieu, Hb dissociates into 32-kDa dimers and 16-kDa monomers. These smaller proteins are freely filtered by the glomerulus, can precipitate in the loop of Henle, and result in severe renal toxicity [35, 44, 45]. This was observed in early studies of Hb-based blood substitutes, and hemoglobinuria was observed in human studies as recently as 1978 [37]. Dissociation of the Hb molecule also decreases its circulating half-life to <1 h as a result of increased renal and haptoglobin–reticuloendothelial system (RES) clearance [3, 46].

Most current HBOC manufacturers have taken similar approaches to this problem by either chemically or genetically cross-linking Hb chains or by polymerizing tetramers (Table 3). The resulting 128-kDa or larger molecules are not readily filtered by the glomerulus, and plasma half-lives are markedly increased [3]. Animal studies report half-lives of 36–48 h for cross-linked or polymerized HBOCs [3], whereas anecdotal reports from human studies suggest half-lives of 3–12 h. Other approaches to eliminate the renal toxicity of Hb include encapsulation [8, 47] or ultrasonically induced microbubbles of Hb [48].

Increased affinity for O2. In addition to eliminating renal toxicity and increasing half-lives, cross-linking or polymerizing Hb also begins to address the problem that in plasma, Hb has a much higher affinity for O2 than it does in the RBC. In solution, the human HbO2 dissociation curve shifts to the left, decreasing the P50 to ~1.33 kPa (10 mmHg) vs a P50 of 3.73 kPa (28 mmHg) in the RBC. With a P50 of 1.33 kPa (10 mmHg), <10% of O2 is released in the periphery at a venous PO2 of 4–5.33 kPa (30–40 mmHg). Such a high-affinity Hb would not function well as an oxygen delivering substance.

The increased affinity for O2 of soluble human Hb is a result of insufficient 2,3-diphosphoglycerate (DPG) in plasma and the more alkaline pH of plasma. 2,3-DPG decreases O2 affinity by cross-linking the β chains of the tetramer and stabilizing the salt bridges present in deoxymoglobin that must be broken for O2 binding [49]. The more alkaline condition of plasma results in less H+ binding to histidine residues, resulting in an allosterically induced higher O2 affinity (the Bohr effect).

Chemically cross-linking either the β or α chains stabilizes the more rigid structure of deoxymoglobin normally maintained by salt bridges and 2,3-DPG, with the net effect of decreasing O2 affinity. Cross-linking is accomplished either chemically or by genetically engineering stable α–α cross-links in recombinant Hb products (Table 3). One product cross-links the ε-amino of the Lys 99 residues of the α chains [50], whereas another fuses two α chains in tandem with a novel DNA construct [51]. O2 affinity in soluble Hb can also be decreased by engineering in mutations such as Hb Presbyterian (β chain, Asn 108 Lys) or a bicarbonate-responsive allosteric site from crocodile Hb [52]. Another approach involves covalently linking pyridoxal phosphate to the 2,3-DPG site [3].

The use of bovine Hb greatly minimizes the affinity problem, as bovine Hb O2 affinity is not 2,3-DPG dependent but rather Cl− dependent [53]. Cl− concentrations are sufficient in human plasma to maintain the allosteric binding properties of soluble bovine Hb. Together these approaches have led to soluble HBOC products with a P50 of 2.93–4.4 kPa (22–33 mmHg), which is similar to that of intracellular human Hb and optimal for peripheral O2 delivery [2].

Vasoactive properties. A significant challenge facing development of HBOCs is their effect on vascular tone as manifested by the hypertension observed in early studies of soluble Hb. Animal studies with current HBOCs continue to demonstrate this vasoconstrictive effect but are also helping to understand the mechanism(s) of this effect [54–57]. Increases in diastolic and systolic pressures of 10–35% peaking 15–30 min after administration and returning to baseline by ~2 h have been observed in some animal studies [55].

Theories regarding the mechanism of the vasopressor effect of soluble Hb products include nitric oxide (NO) scavenging by Hb, excess O2 delivery to peripheral tissues, direct effects on peripheral nerves, or the oxidative properties of Hb [58]. Unlike Hb in RBCs, soluble Hb can be extravasated into endothelial tissue where it can rapidly react with NO and form metHb and NO–Hb [58]. NO, also referred to as endothelial-derived relaxing factor, is a potent endothelial vasorelaxant that inhibits conversion of proendothelin to the vasoconstrictor endothelin [59]. Evidence supporting NO scavenging is provided by studies in rats demonstrating that neither soluble metHb (incapable of binding NO) nor coadministration of an inhibitor of
the proendothelin-to-endothelin conversion resulted in vasoconstriction [55]. Furthermore, coadministration of excess arginine (a precursor of NO synthesis) or nitroglycerine (an NO donor) with soluble Hb did not result in an increase in arterial blood pressure [55]. Some studies suggest that solutions of larger polymerized Hb (either bovine or human) may not exhibit as much vasoconstriction, as they are not as effectively extravasated to the basal side of the endothelium where NO exhibits its physiological activity [60, 61]. Finally, because excess NO is hypothesized to be a major contributor to the hypotension of shock, this property of soluble Hb might actually be advantageous in settings of hypovolemia.

Alternative theories to explain the vasoconstrictive effects of soluble Hb have also been offered [58, 62]. One suggests that without the RBC membrane as a barrier to O₂ diffusion, too much O₂ is delivered to the microcirculation, which responds with an autoregulatory vasocstriction reflex [58]. This effect is suggested to actually be overcome by the use of soluble Hb with a higher O₂ affinity, which runs counter to current thoughts about HBOC development. Direct effects of Hb on peripheral nerves, stimulating noradrenaline production, has also been suggested, because administration of α-adrenergic antagonists minimizes the vasoconstrictive effects of soluble Hb [62]. Finally, oxidation of soluble Hb can result in heme loss, free radical formation, loss of reactive iron, and oxidation of lipids [58]. These reactions and products can result in endothelial stress. RBCs contain catalase and superoxide dismutase (SOD), which counteract these properties of the highly reactive Hb molecule by reversing Hb autooxidation.

The clinical significance of the vasoconstrictive properties of soluble HBOCs in humans is currently unknown, although several non-peer-reviewed accounts of early clinical trials report that hypertension was not observed [63, 64]. Furthermore, as phase II trials of several products are nearing completion and with phase III trials planned, this property likely has not raised significant safety questions. In addition to polymerization that prevents extravasation into the endothelium, other methods under investigation to overcome this property of soluble Hb have focused on genetic engineering [58]. These include modifying cysteine residues on the Hb molecule that are important for NO binding and transport and cross-linking the Hb molecule to SOD or catalase [58].

**Performance.** The HBOC trials currently in progress include use in trauma, ANH, and cardiopulmonary bypass surgery. Although information from the phases I and II safety and dose escalation trials with HBOC products is closely guarded, recent animal data with current HBOC products is promising. For instance, one study in sheep replaced 95% of blood volume with a bovine Hb HBOC product, and these animals showed only minor increases in blood pressure, normal cardiac output, normal venous Po₂, and O₂ content, and recovery to a hematocrit of 20% by day 10 [61]. Similar impressive results have been observed in studies involving other HBOC products in baboons and rats [60, 65]. Preliminary reports of HBOC products suggest few side effects and satisfactory outcomes [63, 64]. These suggest that the use of HBOC products in humans may be possible in the not-too-distant future as one means to minimize allogeneic blood exposure.

**PFCS**

The other type of blood substitute under development and currently in phase I and II trials are PFC based. PFCs are chemically inert, water insoluble, synthetic aromatic or aliphatic compounds with F substituted for all H atoms [66–68]. Initially developed during the Manhattan Project as a chemically inert solvent for handling highly reactive uranium compounds, PFCs are also the basis of several important products such as Teflon® and Scotchguard®. The electron-dense F atoms result in little intramolecular interaction and low surface tension [66], making these molecules excellent solvents for gases. Indeed, some PFC products can dissolve 100 times more O₂ per volume than plasma [67]. Unlike the cooperative binding of O₂ by Hb, the O₂ capacity of PFCs is linearly related to Po₂ and obeys Henry’s law [68]. The first demonstration that PFCs may have clinical utility occurred in 1966 when Clark and Gollan demonstrated that mice fully submerged in oxygenated PFC could survive for hours [69]. Indeed, liquid ventilation with PFCs for pulmonary O₂ delivery in neonates with infant respiratory syndrome [70] and for experimental acute respiratory distress syndrome [71] are two uses that are not discussed here.

The first clinical trials with PFC as an oxygen carrier began in the early 1980s with the Fluosol product from the Green Cross Corp. of Japan [72]. However, the insolubility of PFCs in water necessitates their use as emulsions [66]. One drawback of this product for use as an oxygen carrier was the high viscosity of the emulsion, which limited the concentration of Fluosol that could be infused and thus its capacity to carry and deliver O₂. Even at a Po₂ of 66.6 kPa (500 mmHg), the 20% Fluosol that could be infused contained only 50 mL/L O₂ [73]. In a clinical trial of Jehovah’s Witness patients with acute anemia, Fluosol did not provide sufficient oxygenation to improve outcomes [74]. Nevertheless, Fluosol is the only blood substitute product currently approved by the FDA for clinical use. Fluosol is approved for supplemental oxygenation of ischemic tissue in PTCA because of its ability to diffuse into poorly vascularized tissue [30].

Since the mid-1980s, improvements in the oxygen capacity of PFCs and improvements in the emulsion properties have led to second-generation PFC-based oxygen carriers [75]. These new products can be infused at PFC concentrations as high as 900 g/L without viscosity problems. A PFC product currently in clinical trials is Perfluorbron from Alliance Pharmaceuticals. Perfluorbron (perfluoroocetyl bromide) is an eight-carbon aliphatic mol-
Half-lives, the linear relation of $O_2$ capacity to challenges to routine clinical use. These include short promising properties, the PFC-based substances also face attractiveness as an oxygen carrier Perflubron is stable at 4 °C for up to 4 years, lending to its attractiveness as an oxygen carrier. Despite these promising properties, the PFC-based substances also face challenges to routine clinical use. These include short half-lives, the linear relation of $O_2$ capacity to $P_{O_2}$, and a series of reported side-effects in human volunteers.

**Short half-life.** PFCs are eliminated from the body unmetabolized through the lungs. They are also cleared from the circulation by RES phagocytosis from which they are ultimately excreted by exhalation. This clearance is rapid, with reported half-lives of 2–4 h. The RES clearance also results in slight splenomegaly and hepatomegaly and possibly some other side effects discussed below.

The short half-life of PFCs will likely limit their clinical utility to settings where prolonged volume expansion and restoration of oxygen-carrying capacity is not anticipated. These would include oxygenation of poorly vascularized tissues and tumors, priming of cardiopulmonary bypass pumps, and ANH.

$O_2$ capacity. Probably the most significant limitation for widespread use of PFCs as oxygen carriers is the linear relation of $O_2$ dissolved to the $P_{O_2}$ (Fig. 1). At room air $P_{O_2}$ [18 kPa (~135 mmHg)], the $O_2$ content of 900 mL/L Perflubron is <50 mL/L, whereas at a $P_{O_2}$ of 66.6 kPa (500 mmHg) the $O_2$ content is >160 mL/L (Fig. 1). The latter content is nearly equal to that of whole blood at ambient $P_{O_2}$. Indeed, when a patient is receiving a very high percentage of inspired $O_2$ ($FiO_2$), 100 mL of Perflubron can deliver more $O_2$ to peripheral tissues than 100 mL of whole blood [4, 67]. Perflubron will deliver ~120 mL $O_2$ from an alveolar $P_{O_2}$ of 66.6 kPa (500 mmHg) to a peripheral $P_{O_2}$ of 5.33 kPa (40 mmHg) (Fig. 1). Whole blood with a Hb concentration of 140 g/L will deliver ~100 mL $O_2$ to the periphery whether the alveolar $P_{O_2}$ is 18 kPa (135 mmHg) or 66.6 kPa (500 mmHg) (Fig. 1). However, when the alveolar $P_{O_2}$ is 18 kPa (135 mmHg) (room air), Perflubron will not deliver sufficient $O_2$ for peripheral tissue oxygenation. Thus, to function as an oxygen-carrying volume expander, PFC emulsions require very high $FiO_2$. This property essentially precludes their use in settings where supplemental $O_2$ is not available. The duration of effective use of PFC is thus limited not only by its short half-life but also by the amount of time considered safe for administration of 100% $FiO_2$. Thus, it is likely that PFC use as a blood substitute will be limited to controlled settings when the need for oxygen-carrying supplementation is not expected to be long term.

**Side effects.** Like the HBOC blood substitutes, PFCs are reported to manifest several side effects, the clinical significance of which is not currently fully understood. In healthy, conscious human volunteers a transient "flu-like" syndrome has been described when Perflubron was administered as a contrast agent. Symptoms included back pain, malaise, flushing, and a transient fever of several hours [4, 66, 75]. These symptoms are most likely cytokine-mediated, as the PFC particles are cleared by cells of the RES [77]. One commonly observed side effect is a transient thrombocytopenia 3–4 days after PFC administration [75]. In several studies the mean platelet decrease was 30–40%, with platelet counts returning to normal in 7–10 days [75, 78]. Use of radioactively labeled platelets demonstrated increased platelet clearance that is thought to be due to alteration of the platelet surface by the PFC emulsion. Thus, current PFC products are unlikely to be administered to thrombocytopenic patients.

**Performance.** Despite the reported side effects of PFC-based oxygen carriers, clinical trials are progressing, suggesting that these effects are not severe. As with the HBOC products, phase I and II trials of Perflubron are nearing completion and phase III trials are in planning. Animal studies of Perflubron [76, 79] have been promising, and preliminary data from one human trial has been reported [7]. In one study, the venous $P_{O_2}$ doubled in dogs given 3.3 mL/kg of 900 g/L Perflubron vs control animals given lactated Ringer’s solution, and venous $O_2$ saturation increased 30% in the experimental animals [76]. In the one reported human trial, venous $P_{O_2}$ increased from a mean of 6.4 kPa (48 mmHg) to 7.73 kPa (58 mmHg) with Perflubron administration after ANH in eight patients [7]. As phase II trials near completion, further reports of clinical experience can be expected.

A safe and effective substitute for the oxygen-carrying role of blood has been a goal for nearly as long as blood transfusion has been practiced. Today’s climate of public

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**Fig. 1.** Comparison of oxygen capacity of whole blood, HBOC, soluble unmodified human Hb at 7 g/L (dotted line), and two PFC blood substitutes vs oxygen tension.

uneasiness concerning the safety of human-derived blood products, combined with a projected shortage of blood products as the baby-boom generation ages, has heightened interest in such products. Until relatively recently, however, no practical oxygen carriers have been available. In the last few decades, much progress has been made in improving both the safety and efficacy of blood substitutes. Improvements include lengthened half-life, improved oxygen delivery, and reduced side effects. This is true for both the Hb- and the PFC-based products. Although none of these products is likely to be a viable substitute for RBCs in situations where long-term oxygen-carrying capacity is needed, they may have a clinical role as a short-term, oxygen-carrying volume-expanding fluid. Likely indications include ANH, emergency replacement of oxygen-carrying capacity after trauma (both civilian and military), and novel uses such as improving oxygen delivery to ischemic tissues due to the small size of these oxygen carriers compared with RBCs. As these products move toward phase III trials, it is important for the laboratory medicine community to begin thinking about and preparing for potential analytical challenges associated with their use [80, 81].

References

18. Skolnick AA. As the blood supply gets safer, experts still call for ways to reduce the need for transfusion. JAMA 1992;268:698–700.


