Red blood cell substitutes: current status

J. A. Jones

There are two main indications for the transfusion of red cells: severe haemorrhage and chronic symptomatic anaemia for which no specific therapy exists. In both circumstances, the aim of red cell transfusion is to improve the oxygen supply to the tissues by raising the oxygen content of the blood, according to the equations:

\[
\text{oxygen delivery} = \text{cardiac output} \times \text{arterial oxygen content}
\]

\[
\text{arterial oxygen content} = \text{haemoglobin concentration} \times \% \text{saturation} \times 1.34 \quad [34]
\]

The transfusion of allogeneic blood (or plasma or red cells) involves many hoards (see table 1), some of which may be reduced or avoided. For example, the patient's own blood rather than allogeneic blood may be transfused: by 1990 more than 5% of units collected in the USA were of autologous blood [42]. In the United Kingdom, the National Transfusion Service is now, in some regions at least, prepared to organize preoperative donation of the patient's blood. Other forms of autologous transfusion, intraoperative haemodilution and red cell salvage, are already practised in the UK. Finally, a growing awareness of the compensatory mechanisms which, in anaemia, preserve oxygen delivery despite a lowered oxygen-carrying capacity, has prompted surgeons and anaesthetists to accept haemoglobin concentrations as low as 7 g dl⁻¹ in some patients.

Compensatory mechanisms in anaemia

Experiments in animals and healthy volunteers have shown that, in acute normovolaemic anaemia induced by withdrawing blood and replacing it with dextran 70, oxygen delivery is at its peak, not at the normal haemoglobin concentration of about 13 g dl⁻¹, but at 10 g dl⁻¹ (see fig. 1). Two mechanisms, both induced by the lowered haemoglobin concentration, are believed to be responsible: an increase in cardiac output and a reduction in blood viscosity, which enhances peripheral flow [28]. More recently, attention has focused on the fact that, in acute normovolaemic anaemia at a haemoglobin concentration of 7 g dl⁻¹, the same compensatory mechanism should produce an oxygen delivery equal to that obtained at a haemoglobin concentration of 13 g dl⁻¹.

In chronic anaemia, another compensatory mechanism comes into play. An increased concentration of 2,3-diphosphoglycerate in red cells causes a shift of the oxygen dissociation curve to the right, enhancing release of oxygen to the tissues. Not all patients have the cardiac reserve required to compensate for a reduction in haemoglobin concentration, but clinical experience in patients with severe anaemia caused by renal failure, and clinical studies in anaemic [44] and in haemodiluted [29] patients subjected to surgery, suggest that many of these patients can be managed safely without transfusion.

Nevertheless, the prospect of a safe, effective, red cell substitute remains appealing. A cell-free, oxygen-carrying solution with a long shelf-life would require no cross-matching (and hence save laboratories' time) and could be instantly available in the casualty department or on the battlefield. The red cell substitutes studied to date are perfluro compounds and haemoglobin solutions and, of these, haemoglobin solutions have been the subject of more extensive recent research.

Haemoglobin solutions

Solutions of haemoglobin would seem to be a natural substitute for red cells, and it has been known for many years that animals survive complete exsanguination if they are transfused with haemoglobin solution [1], but not if they are given a plasma substitute alone. Haemoglobin solutions which are contaminated by red cell debris are toxic to humans [38], so any product intended for human use must be stroma-free. However even pure solutions of human haemoglobin present serious problems.

First, extracellular haemoglobin disappears rapidly from the circulation. Free haemoglobin combines with the plasma protein, haptoglobin, and the haemoglobin–haptoglobin complex is cleared (with a half-life of 10–30 min) into the mononuclear phagocyte system. When haptoglobin is exhausted, about half the remaining free haemoglobin is excreted rapidly by the kidneys [6]. The molecule of haemoglobin A (molecular weight 65 kD) is a tetramer of four globin chains, two alpha and two beta, each attached to a haem prosthetic group.

Key words


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Table 1 Hazards of blood transfusion

<table>
<thead>
<tr>
<th>Hazard</th>
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<tbody>
<tr>
<td>Red cell incompatibility</td>
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<tr>
<td>Febrile reactions</td>
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<tr>
<td>Transmission of infections</td>
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<td>From donor</td>
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<td>By contamination on storage</td>
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<tr>
<td>Circulatory overload</td>
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<td>Thrombophlebitis</td>
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<td>Special hazards of massive transfusion</td>
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<tr>
<td>Hypothermia</td>
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<tr>
<td>Metabolic—citrate intoxication</td>
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<tr>
<td>Dilution of clotting factors</td>
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<tr>
<td>Microaggregates</td>
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<td>Shift of oxygen dissociation curve</td>
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</tbody>
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Figure 1 Relationship of oxygen delivery (systemic oxygen transport capacity) to packed cell volume (PCV).

When outside the red cell, however, the tetrameric molecule dissociates into two alpha-beta dimers, each with a molecular weight of 32 kD, and these are easily able to pass through the renal glomeruli (half-life of plasma clearance less than 1 h). Any haemoglobin not filtered is cleared, in common with the haemoglobin–haptoglobin complex, by the mononuclear phagocyte system [5].

Second, the oxygen affinity of human haemoglobin in solution ($P_50$ less than 1.3 kPa) is far greater than that of intracellular haemoglobin ($P_50 = 3.7$ kPa) (fig. 2). The avidity of extracellular haemoglobin for oxygen is accounted for by two factors, the loss of 2,3-diphosphoglycerate with the red cell stroma, and the relatively alkaline pH of plasma in comparison with the interior of a red cell (Bohr effect).

There are several lesser problems. Haemoglobin solutions have a high colloid osmotic pressure which usually prohibits their administration at concentrations greater than 7 g dl$^{-1}$ [32]. Haemoglobin in solution is gradually oxidized to methaemoglobin and so must be stored in an oxygen-free environment [47]. Finally, large-scale manufacture of a haemoglobin product from human red cells is not really feasible as blood donors are in short supply.

Figure 2 Oxygen dissociation curves of extracellular (E) and intracellular (I) haemoglobin.

Table 2 Products undergoing phase I trial

<table>
<thead>
<tr>
<th>Substance (Manufacturer)</th>
<th>Half-life in circulation (large mammals)</th>
<th>$P_{50}$ (kPa)</th>
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</thead>
<tbody>
<tr>
<td>Poly SFH-P (Northfield)</td>
<td>40–46 h</td>
<td>2.9–3.2</td>
</tr>
<tr>
<td>Bovine (Biopure)</td>
<td>40–46 h</td>
<td>2.9</td>
</tr>
<tr>
<td>DCLHb (Baxter)</td>
<td>36 h</td>
<td>3.9</td>
</tr>
<tr>
<td>Recombinant (Somatogen)</td>
<td>Not established</td>
<td>4.4</td>
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Several products which, to some extent at least, overcome the two main difficulties have been tested extensively in animals, and it is known that four have undergone phase I trials in humans (table 2). It is difficult to obtain up-to-date information in this area because, for proprietary reasons, data are jealously protected and reports in scientific journals are few.

PROLONGING THE HALF-LIFE OF HAEMOGLOBIN IN THE CIRCULATION

The aim of all of the techniques discussed below is to produce a molecule which does not dissociate into alpha-beta dimers and pass through the renal glomeruli.

Polymerization

Glutaraldehyde has been used to form intermolecular bridges between haemoglobin tetramers, the resulting polymers ranging from two to six tetramers (molecular weight 130–400 kD), with a half-life in the circulation of 40–46 h. A haemoglobin solution of a smaller number of larger molecules at any given concentration (w/v) has the advantage of a lower colloid osmotic pressure, so that higher concentrations of haemoglobin may be used without causing a shift of water from the extravascular to the intravascular compartment. Two of the four products which have been tested in humans are polymerized haemoglobin solutions.

Poly SFH-P (Northfield Laboratories) is a polymerized human haemoglobin solution, which is
Red blood cell substitutes

pyridoxalated before polymerization. Pyridoxal phosphate binds covalently at the same site on the beta chains as does 2,3-diphosphoglycerate [2], and thus lowers the oxygen affinity of the haemoglobin molecule (P 50 increased to 2.9–3.2 kPa). At a concentration of 14 g dl −1, Poly SFH-P has been demonstrated to be an effective oxygen carrier which supports life in baboons in the absence of red cells [16]. Preliminary tests in human volunteers were initially abandoned after one of the six subjects developed a “mild allergic reaction” [31], but, in a second series of 27 volunteers infused with Poly SFH-P at doses of 0.125–0.6 g kg −1 (giving plasma concentrations of 0.25–1.2 g dl −1), no adverse effects were observed. A further trial of Poly SFH-P is now in progress in patients, using doses of at least 50 g. No adverse effects have been reported to date; more than 20 patients have been given Poly SFH-P [35].

**Bovine haemoglobin**, unlike human haemoglobin, does not bind 2,3-diphosphoglycerate [4], and relies on chloride ions to reduce its oxygen affinity. The chloride ion concentration of human plasma is sufficient to keep the P 50 of bovine haemoglobin at 2.9 kPa before polymerization [14]. Polymerized bovine haemoglobin (Biopure) has been shown to be a safe and effective carrier of oxygen in sheep [50]. A similar product has been used in the management of aplastic and vaso-occlusive crises in Africans with sickle cell disease [12]. A bovine haemoglobin product would have attractions as a red cell substitute. The source is readily available for large-scale manufacture, and is free from human viruses. These advantages are counterbalanced by uncertainties about its possible antigenicity and fears of bovine spongiform encephalitis (mad cow disease) [7]. A safety study of Biopure’s product in volunteers in the USA was abandoned because of unspecified “medical events” [35].

**Internal stabilization of the tetrameric molecule**

The intravascular half-life of extracellular haemoglobin may be prolonged by preventing dissociation into alpha-beta dimers by the formation of linkages between either the alpha or the beta chains of the tetrameric structure. Two different methods of making such linkages have yielded further haemoglobin preparations for trial in humans.

**Dibromosalicylbisfumarate linkage (DCLHb Baxter Healthcare).** Fumarate bridges may be formed between either the alpha or beta globin chains. A tetramer stabilized by alpha-alpha cross-linkage has a higher P 50 (3.9 kPa) than one with beta-beta bridges, and a plasma half-life in the pig of 36 h [9]. Alpha-alpha cross-linked haemoglobin, prepared from time-expired human blood, was developed at the Letterman Army Institute of Research (LAIR) in San Francisco and was studied there in pigs at the time of the Gulf War. Pigs were shown to survive without red cells if the cells were replaced with an equivalent volume of haemoglobin [19], and a resuscitation study, designed to simulate battlefield injury in dehydrated soldiers, was also carried out. When administered in doses ranging from 0.025 to 0.1 g kg −1 to 24 human volunteers, DCLHb was found to have a disappointingly short half-life of 1.5–3 h in the circulation. The recipients showed a dose-related increase in mean arterial pressure, with an associated reduction in heart rate, which reached a peak at 2–3 h, but returned to normal by 12 h. DCLHb was otherwise tolerated well, and safety studies are being conducted in a wide range of patients [35].

**Genetically engineered haemoglobin (rHb1.1 Somatogen).** In 1990, it was established that fully synthetic human haemoglobin could be prepared, using recombinant DNA technology, in Escherichia coli [20] and yeast [51]. Attention was then turned to modifying the molecular structure to prolong its plasma half-life and increase its P 50. A significantly longer retention time (in rats) was achieved by fusing the alpha chains in tandem, C terminus to N terminus. Taking advantage of the knowledge that an abnormal human haemoglobin, Hb-Presbyterian, has a P 50 of 4.4 kPa, the relevant mutation (A Sn 108 α Lys) was induced in the beta chains to achieve the same effect [26]. This product does not appear to be nephrotoxic in dogs. Safety studies, using rHb1.1 0.015–0.32 g kg −1 in 76 human volunteers, also demonstrated no renal damage, but a transient increase in arterial pressure was observed at the larger doses. Some volunteers had gastrointestinal side effects, which subsequent studies have shown to be caused by altered smooth muscle function. Safety tests are now being performed in anesthetized surgical patients [35].

In recent years it has been possible to prepare transgenic mice, 80% of whose haemoglobin is human [18,39]. Functional human haemoglobin has also been produced in transgenic pigs [45]. If transgenic large animals could be induced to produce genetically engineered human haemoglobin variants such as the one described above, a large-scale source of “optimized” human haemoglobin would become available.

It is worth mentioning two further possible ways of prolonging the half-life of extracellular haemoglobin in the circulation.

**Macromolecular linkage**

Haemoglobin covalently linked to dextran or hydroxyethylstarch has a significantly longer survival than “free” haemoglobin, although the oxygen affinity of these preparations is unfavourably high [46].

Encapsulation

Haemoglobin may be enclosed in lipid vesicles or liposomes. There is no doubt that these “pseudoerythrocytes” support animals in survival studies [11], but the survival time of existing preparations is short [21]. However successful the current phase I trials may
nitric oxide has been identified as the endothelium-postulated that, when free from red cells, haemoglobin solutions showed a persistently low cardiac output and arterial pressure rapidly returned to pre-haemorrhage values. Animals given haemoglobin solutions showed a persistently low cardiac output, with an increase, well above the original level, in systemic and pulmonary artery pressures, accompanied by an increase in systemic and pulmonary vascular resistance. The mechanism underlying the vasoactive effect associated with the administration of haemoglobin solutions in the LAIR resuscitation study is obscure. It has, however, been known for many years that haemoglobin may cause vasoconstriction as well as by inhibition of nitric oxide [41].

**Interference with macrophage function**

Because extracellular haemoglobin is, to a large extent, cleared by the mononuclear phagocyte system, it is reasonable to suspect that haemoglobin may block the system and thus possibly interfere with essential functions such as ingestion of bacteria. This suspicion is supported by the finding that an injection of *Escherichia coli* caused fatal septic shock in animals pretreated with haemoglobin, but not in those given haemoglobin after bacterial infection [24]. It has also been suggested that haemoglobin may stimulate macrophages to release thromboxane and hydrogen peroxide [43].

**TRIALS OF EFFICACY**

In phase I trials, where only very small doses of the agent in question are administered, safety rather than efficacy is tested. After satisfactory phase I trials of a product, tests of its efficacy (phase II trials) may be planned. The design of such studies is proving to be difficult. Ethical considerations preclude testing the efficacy of haemoglobin solutions against placebo in patients with severe bleeding, because it is known that an effective treatment, namely the transfusion of red cells, already exists. It has been suggested that red cell substitutes might be tested in the initial resuscitation of severely injured patients outside hospital where red cell are not available but, in the United States, the Center for Biologies Evaluation and Research (CBER) regards such studies as "premature at this time". The CBER does, however, consider that trials of red cell substitutes might be feasible in acutely bleeding patients in intensive care units [13].

It has also been suggested that efficacy studies might ethically be made of the effect of red cell substitutes on oxygen delivery and consumption in the circulation.

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**Table 3 Side effects of haemoglobin solutions**

<table>
<thead>
<tr>
<th>Vasoactivity</th>
<th>Nephrotoxicity</th>
<th>Interference with mononuclear phagocyte system</th>
<th>Antigenicity</th>
<th>Oxidation on storage</th>
<th>Activation of complement, kinin and coagulation</th>
<th>Histamine release</th>
<th>Iron deposition</th>
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prove, further questions need to be answered. Are haemoglobin solutions safe in therapeutic doses? Are they clinically effective and, if so, in what circumstances? Partial answers may be given, even now, to these questions.

**UNWANTED SIDE EFFECTS OF HAEMOGLOBIN SOLUTIONS**

The list of possible side-effects of haemoglobin solutions is a long one, as shown in table 3. The possibility, even though remote, that bovine haemoglobin may prove immunogenic and the fact that haemoglobin in solution is gradually oxidized to methaemoglobin unless stored in oxygen-free conditions, have already been mentioned.

**Vasoactivity**

It has already been pointed out that acute normovolaemic haemodilution with crystalloid or colloid solutions is accompanied by an increase in cardiac output. If blood removed from experimental animals is replaced with Poly SFH-P or with polymerized bovine haemoglobin, cardiac output remains constant, as do arterial oxygen content and oxygen delivery to the tissues [17,50]. These experiments suggest that haemoglobin solutions are innocuous to the circulation.

The LAIR resuscitation study [52] however, suggested that both cross-linked DCLHb and uncross-linked haemoglobin might be vasoactive. In this study, pigs were used as a model of desert battlefield casualties [52]. After dehydration for 2 days, 30% of the pigs' blood volume was removed, and resuscitation was carried out using Ringer's acetate, albumin, alpha-alpha cross-linked haemoglobin or an uncrosslinked haemoglobin solution. In the animals resuscitated with plasma substitutes, cardiac output and arterial pressure rapidly returned to pre-haemorrhage values. Animals given haemoglobin solutions showed a persistently low cardiac output, with an increase, well above the original level, in systemic and pulmonary artery pressures, accompanied by an increase in systemic and pulmonary vascular resistance. The mechanism underlying the vasoconstrictor peptide endothelin as well as by inhibition of nitric oxide [41].

**Nephrotoxicity**

It is known that elements of red cell stroma damage the kidneys [38]. Infusions of tetrameric, stromalr-free haemoglobin have been shown to cause renal damage in human volunteers [40] and baboons [43]. It is uncertain if impurities or alpha-beta dimers themselves were responsible for the nephrotoxicity in these species. The products at present undergoing phase I trials in humans seem to be devoid of renal toxicity in the animal studies already mentioned, as does haemoglobin bound to dextran.

**Interference with macrophage function**

Because extracellular haemoglobin is, to a large extent, cleared by the mononuclear phagocyte system, it is reasonable to suspect that haemoglobin may block the system and thus possibly interfere with essential functions such as ingestion of bacteria. This suspicion is supported by the finding that an injection of *Escherichia freundii* caused fatal septic shock in animals pretreated with haemoglobin, but not in those given haemoglobin after bacterial infection [24]. It has also been suggested that haemoglobin may stimulate macrophages to release thromboxane and hydrogen peroxide [43].
patients (or volunteers) subjected to acute normovolaemic haemodilution [53]. Because acute anaemia is well tolerated by healthy people provided the circulating volume is maintained, it may be difficult to design studies to define the point at which haemoglobin solutions are better than plasma substitutes alone. There is no precise answer to the question of how much oxygen is enough.

**Perfluoro compounds**

Mice have been demonstrated to survive immersion in perfluorocarbon (PFC) through which oxygen is bubbled [8]. Although oxygen is highly soluble in perfluoro compounds, these compounds have great limitations.

Perfluoro compounds are insoluble in water. They have to be emulsified and stored in the frozen state. The emulsions are viscous, which implies that only a low plasma concentration can be achieved. Perfluoro compounds are cleared rapidly from the circulation and are retained in the mononuclear phagocyte system [36]. They are excreted unchanged through the lungs over about 7 days. There is evidence that macrophages which have ingested perfluoro compounds show loss of phagocytic function, and it is also suspected that macrophages may be induced to release cytokines and other immune mediators [48]. The emulsifying agents may activate complement and cause pulmonary damage [49].

The greatest physiological limitation of perfluoro compounds is that the amount of oxygen they dissolve is related linearly to partial pressure. Clinically significant amounts of oxygen can be carried only if the arterial (and hence inspired) oxygen partial pressure is high, and prolonged breathing of high oxygen concentrations may be toxic. However, animals have been shown to survive total blood replacement with perfluorocarbons. Rats in which a perfluoro compound known as FC-80 was substituted for blood were initially in 95% oxygen but the inspired oxygen concentration was reduced progressively as red cells and serum proteins were regenerated, so that the animals were returned to air at 7 days; some of them were alive 1 yr later [15].

**FLUOSOL-DA**

A 20% emulsion of two different perfluoro compounds, described as Fluosol-DA, which has an oxygen-carrying capacity at 37 °C of approximately 40% of that of red cells, was given as an i.v. infusion, at a dose of about 20 ml kg⁻¹, to 186 patients. Side effects were observed in only one patient who was given repeated infusions [30]. No serious harm seems to have come to any patient who has received Fluosol-DA.

As a blood substitute, Fluosol-DA was found to be ineffective as an oxygen carrier in human surgical patients with acute severe anaemia who had refused blood transfusion on religious grounds [16]. Fluosol-DA has, however, found a place in more restricted circumstances. Oxygenated Fluosol-DA was shown to prevent myocardial ischaemia when infused into the distal coronary artery during balloon angioplasty, whereas infusions of oxygengated Ringer’s lactate solution and non-oxygenated Fluosol-DA conferred no such benefit [22]. In 1990, the use of Fluosol-DA in coronary angioplasty was approved by the FDA. By improving tissue oxygenation, Fluosol-DA may also prove an adjuvant to radiotherapy. Clinical trials are in progress [27].

**POLYFLUORO-OCTOBROMIDE (PERFLUBRON)**

This new perfluoro compound, which is radiopaque, has two advantages over its predecessors. First, higher concentrations of perfluoro compound may be administered, because a 100% (w/v) emulsion with phospholipid has a sufficiently low viscosity to be infused without dilution. Second, oxygen is more soluble in polyfluoro-octobromide than in any other perfluoro compound introduced to date [25]. It has been calculated that, at a PO₂ of 37 kPa, polfluoro-octobromide can carry as much oxygen as a haemoglobin solution at a concentration of 7 g dl⁻¹. Arterial oxygen partial pressures of this order should be achieved in patients with normal lungs at an FIO₂ of 50–60% (see fig. 3). Perflubron has more promise as an oxygen carrier in clinical practice than any perfluoro compound introduced so far. It has been proposed that Perflubron might find a place in intraoperative haemodilution [10], and efficacy testing in this area has been started in humans [K. K. Tremper, personal communication]. Polyfluoro-octobromide may prove to be of use as an oxygen carrier in resuscitation and in intraoperative haemodilution. A wide range of non-transfusional uses has been suggested [3].

**Conclusion**

The red cell substitutes under trial at present have far too short a survival time in the circulation to be substitutes for red cells in the treatment of chronic anaemia. It is much easier to envisage a role in short-term procedures, such as immediate resuscitation of military or civilian casualties. Of course, red cells would have to be transfused within the next 24 h. It must be pointed out that oxygen transport is the only...
function of red cells which has been considered in this review. Red cells do, however, have other important roles, most notably in carbon dioxide carriage and in buffering. Extracellular haemoglobin is able to form carbamino compounds and bind hydrogen ions, and carbon dioxide transport has been reported to be normal in animal studies of the four haemoglobin solutions at present undergoing phase 1 trials. It is difficult to predict that carbon dioxide transport would remain normal if haemoglobin without carbonic anhydrase or perfluoro compounds were used to resuscitate hypovolaemic patients.

Oxygen-carrying substances may also have a role in haemodiluted patients undergoing elective surgery. The administration of a red cell substitute might enable more blood to be withdrawn from patients at the beginning of their operation and tide them over the period of haemodilution before the blood was re-transfused. Avoidance of allogeneic blood transfusion would not only be beneficial for the individual patient, but if the practice became widespread it would help relieve pressure on transfusion services for ever increasing quantities of allogenic blood [23].

Considerable ingenuity has been applied to modify both haemoglobin and perfluoro compounds to produce agents which may prove valuable as short-therapy. The administration of a red cell substitute safe, effective product emerges, it will be aggressively invested in the search for a red cell substitute. If a both haemoglobin and perfluoro compounds to allogenic blood [23].

References


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