Synthetic Blood

SUPPLY FROM A DIFFERENT VEIN

By Bruce Goldfarb

Medical science has created ingenious replacements for a wide range of body parts: artificial kidney, artificial heart, synthetic blood vessels and joints, eye and ear implants. But there's still no substitute for blood. The five liters of blood pumping through the average adult body serve several vital roles. Blood transports oxygen and carbon dioxide, acts as the body's sewage system, wards off infectious disease, and has a clotting system to seal leaks. Developing a single synthetic blood product that does all these functions may be possible one day, but that day is may be decades from now.

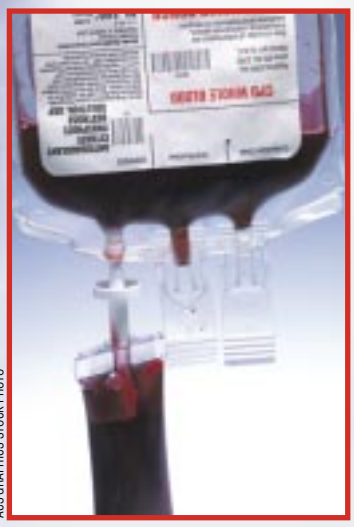
Scientists have devised the next-best thing: oxygen-carrying blood substitutes. Several of these remarkable fluids are undergoing clinical trials in humans and may be approved by the U.S. Food and Drug Administration within one or two years.

Until the mid-1980s, research in synthetic blood was viewed with doubt and amusement within the scientific community. "People who worked on artificial blood were curiosities," says Leland Clark, a chemist at Antioch College, Yellow Springs, OH.

The emergence of the AIDS virus—and the risk of spreading lethal disease through donated blood—brought the need for a substitute into sharp focus. A screening test for AIDS was introduced in 1985. Although blood screening is reliable, it is not perfect. The chances of passing the AIDS virus by blood are extremely small; however, the risk is anything but trivial if you're on the receiving end of one of the 10 million units of blood transfused annually in the United States.

A synthetic blood substitute would be sterile and completely free of disease. It could be given without the need for blood typing and cross-matching. The ideal blood substitute wouldn't require refrigeration or special handling. Most importantly, it would eliminate the shortages that plague the blood supply.

More than a dozen companies are racing to bring synthetic blood substitutes to the market, a market estimated to be worth billions of dollars. There are two basic varieties of blood substitute—one is based on hemoglobin, the iron-containing, oxygen-carrying molecule, and the other uses oily substances that are liquid Teflon-like compounds known as perfluorocarbons.



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Artificial cells

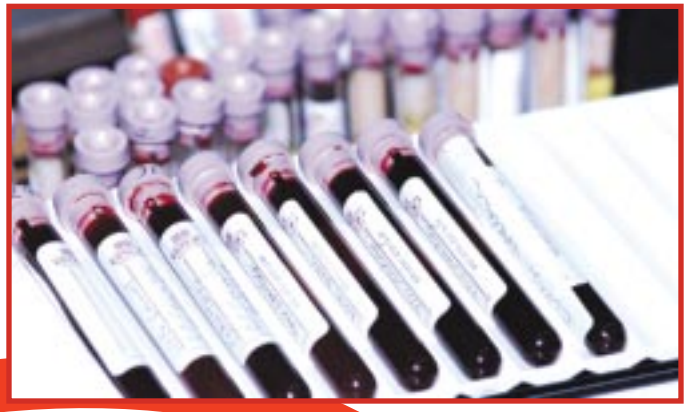
Creating a hemoglobin-based blood substitute is a formidable scientific challenge. Problems arise when hemoglobin is removed from its protective red cell membrane. The membrane envelope protects the molecule from degradation in the bloodstream. Equally important, the cell membrane prevents hemoglobin from turning into a toxic poison.

"Raw hemoglobin extracted from red blood cells cannot be used as a blood substitute," explains Thomas Chang of McGill University in Montreal, Canada. Chang has been researching artificial cells and synthetic blood for more than 30 years.

Each hemoglobin molecule is a tetramer composed of four subunits that break into half-molecule dimers when liberated from red cells. If injected into the body, the dimers quickly cause severe kidney damage and other toxic effects. To avoid these problems, hemoglobin is modified by cross-linking into larger, nontoxic molecules. A half-dozen companies are developing varieties of cross-linked or conjugated hemoglobin—some from human sources, some based on hemoglobin from cows, and some using recombinant DNA technology.

One company is testing a blood substitute containing hemoglobin derived from stored human blood that is past its expiration date. The hemoglobin molecules are glued together with diaspirin, a derivative of aspirin. Another company has begun human testing with a blood substitute that has four or five molecules strung together into polyhemoglobin.

The first batch of hemoglobin-based blood substitutes remains in the body for 20–30 hours and is useful only on a short-term basis. "Good enough to sustain you, but only for a day or so," says Chang. "The first generation [of hemoglobin-based blood substitutes] is now well into clinical trials, and one or two of them may be in routine use very quickly, in a year or two."



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White blood

A second variety of blood substitute is based on perfluorocarbons, which have an enormous capacity to dissolve oxygen—50 times greater than blood plasma. According to Clark, a 100-mL beaker of pure perfluorocarbon can hold 40–50 mL of oxygen.

Perfluorocarbons are among the most inert materials ever discovered. Teflon, which is a perfluorocarbon, reacts with hardly anything. Teflon-coated pots and pans have been in American homes since the 1960s.

Perfluorocarbons have been used for more than 30 years as a sort of scientific parlor trick to demonstrate liquid-breathing animals (see sidebar, "A Womb with a View"). Now liquid breathing is gaining interest as a life-saving treatment for premature babies and others with acute respiratory distress.

To be injected into the bloodstream, perfluorocarbons must be emulsified (dispersed in a suspension), which gives the liquid a milky white appearance. Phospholipids from egg yolk are used to emulsify perfluorocarbons into particles about 0.12 μm across—1/70th the size of red blood cells. Perfluorocarbon is an ideal blood substitute because it does not react in the body and slowly evaporates out of the bloodstream through the lungs.

The first perfluorocarbon-based blood substitute, Fluosol DA (perfluorodecalin), was introduced in 1978 by the Japanese company Green Cross. The "white blood" was given to about 2000 volunteers, Clark says, primarily Jehovah's Witnesses and other people who are unable to receive blood products because of their religious beliefs. Fluosol was also approved to maintain perfusion (the flow of oxygen through the arteries) to the heart during balloon angioplasty, a procedure that widens blocked heart arteries.

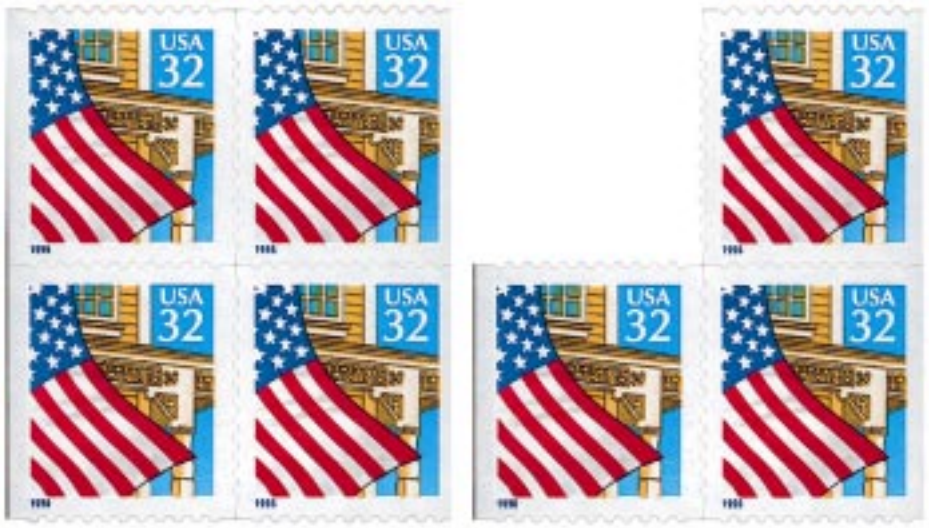
Other companies are also developing perfluorocarbons. Alliance Pharmaceutical produces perflubron (perfluorooctylbromide) under the brand name Oxygent. Alliance markets the liquid-breathing fluid LiquiVent with Hoechst Marion Roussel.

Tests in humans are under way to study the use of perflubron during surgery. Before surgery, doctors drain about one-third of a patient's blood and store it in a refrigerator. The patient is given perflubron and saline solutions to compensate for the fluid loss and maintain safe oxygen levels during surgery. The patient's own blood is returned when the surgical procedure is nearing completion.

Because perfluorocarbons evaporate from the bloodstream through the lungs, vapor pressure is a key factor for identifying a liquid that is safe and effective. Vapor pressure that is too high causes lethal lung hyperinflation. If the vapor pressure is too low, the perfluorocarbons will remain in the body for a very long time, perhaps accumulating in the liver or other tissues.

"There must be some vapor pressure that is such so it [perfluorocarbon] won't evaporate too fast and hyperinflate the lung or evaporate so slowly that it stays in the body forever," says Clark. "It's taken the past 10 years of my life to figure out which is the right one."

Boiling point is a convenient function of vapor pressure. "Perfluorodecalin has a boiling point of about 140 °C with a vapor pressure which is too high and causes



Hemoglobin

The hemoglobin molecule is composed of four subunits; each subunit can bond one O_2 molecule. As each molecule of oxygen bonds, it changes the shape of the hemoglobin molecule, making it easier to bind another oxygen molecule. The more O_2 molecules attached, the easier it is to add another. Losing O_2 works the same way; the more O_2 lost, the easier it is to lose more. This is important because it must be easy for the blood to lose O_2 in the tissues of the body where it is needed and to gain O_2 from the lungs where it is available. This property makes hemoglobin much more efficient (1.8 times) in carrying O_2 than a solution that simply dissolves O_2 gas.

This principle is similar to using a block of postage stamps. To use the first stamp, two perforated edges must be torn. The next stamp is easier to remove since only one edge must be torn.

A Womb with a View

Perfluorocarbon can be used to deliver oxygen directly through the lungs, allowing humans to breathe liquid like a fish. For a dramatic portrayal of liquid-breathing perfluorocarbon technology, view the 1989 film, *The Abyss*.

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In the case of liquid breathing, truth is stranger than fiction. In the early 1960s, physiologist Johannes Kylstra did experiments in Holland and at the University of Buffalo (NY) in which mice breathed oxygenated water in a hyperbaric chamber for up to 18 hours, but the animals often died soon after being removed.

The prospect of a self-contained liquid-breathing system that would eliminate the problems of "the bends" and nitrogen narcosis during deep sea dives was of interest to the U.S. military. Leland Clark, inventor of the heart–lung machine, was on the faculty of the University of Alabama Medical College in Birmingham when he heard about Kylstra's work. In 1963, he began to wonder if some other kind of liquid would work better than water. "I was thinking about the high solubility of oxygen in silicone oil," Clark recalls. "We used silicone oil to coat parts in the [heart–lung] machine. Silicone oil is very inert, and it dissolves about 20% oxygen, just like air."

In his lab, Clark bubbled oxygen through a beaker of silicone oil and dunked a startled rat beneath the surface. The rat breathed silicone oil for 20 minutes, remaining a healthy pink the whole time. "I don't know who was more surprised, myself or the rat," says Clark. Soon he had goldfish surviving in silicone oil for weeks.

The search for a fluid medium to dissolve oxygen led Clark to perfluorocarbons, which "hold a huge amount of oxygen." Unlike the emulsion of synthetic blood, the perfluorocarbon used for liquid breathing is "neat," without surfactant or other additives.

To allow liquid breathing, a tube is inserted into the patient's airway, which is then sealed with an inflatable cuff. The patient's lungs are filled with perfluorocarbon that is pumped through a mechanical liquid ventilator. Alternatively, a space may be left in the upper airway that is ventilated with 100% oxygen via a standard mechanical ventilator.

Research shows that perfluorocarbon liquid breathing may be life-saving for premature babies, who are born before their lungs are fully developed. Annually about 80,000 premature babies in the United States have severe breathing problems.

At Temple University (Philadelphia, PA), Thomas Shaffer and colleagues put critically ill premature babies on liquid breathing for up to 19 hours with no apparent ill effects. "We all spend nine months . . . with our lungs filled with fluid," says Shaffer. "What we're trying to do, to a certain extent, is to reproduce that fluid-filled lung environment."

Liquid breathing could also help critically ill victims of smoke inhalation, near-drowning, severe infections, and adult respiratory distress syndrome. According to Clark, each year in the United States, about 300,000 hospitalized patients require mechanical ventilation.

hyperinflation. If you put a methyl group on perfluorodecalin, it boils at 160 °C. That [vapor pressure] is too low to evaporate fast enough. It would stay in the body for at least a year," Clark says. "So between those two boiling points there should be some [perfluorocarbons] that are just about right, at around 150 °C. These are the ones we've been working on, and we've found more than one." Clark's company, Synthetic Blood International, could have candidate perfluorocarbon, oxygen-delivery, blood substitutes in human tests within 2 years.

Research continues by Clark and others to discover the first successful substitute that uses either the hemoglobin or the perfluorocarbon model. Perhaps, in the near future, multifunction blood substitutes will not be that far-fetched for use in the ER and surgery, as protection from blood-borne diseases, as a treatment for oxygen-depravation cases (especially to relieve the agony of the bends), and for use by people whose religious convictions prevent them from having blood transfusions.

Bruce Goldfarb is a science and medical writer in Baltimore, MD. His article "Color in a Capsule" appeared as a feature article in the February 1998 issue of *ChemMatters*.

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