Sanguine Substitutes

Years of research confirm the difficulties of concocting better blood

By RICK WEISS

Ancient Hindus called it the embodiment of wisdom. To the Egyptian pharoahs it was an elixir of immortality, and to the bacchanalian Greeks it was supernatural red wine.

It has been drunk by pagan priests at sacrificial ceremonies and smeared over the bodies of warriors before battle. Even in this age of scientific rationality, the mere sight of it is enough to make grown men faint.

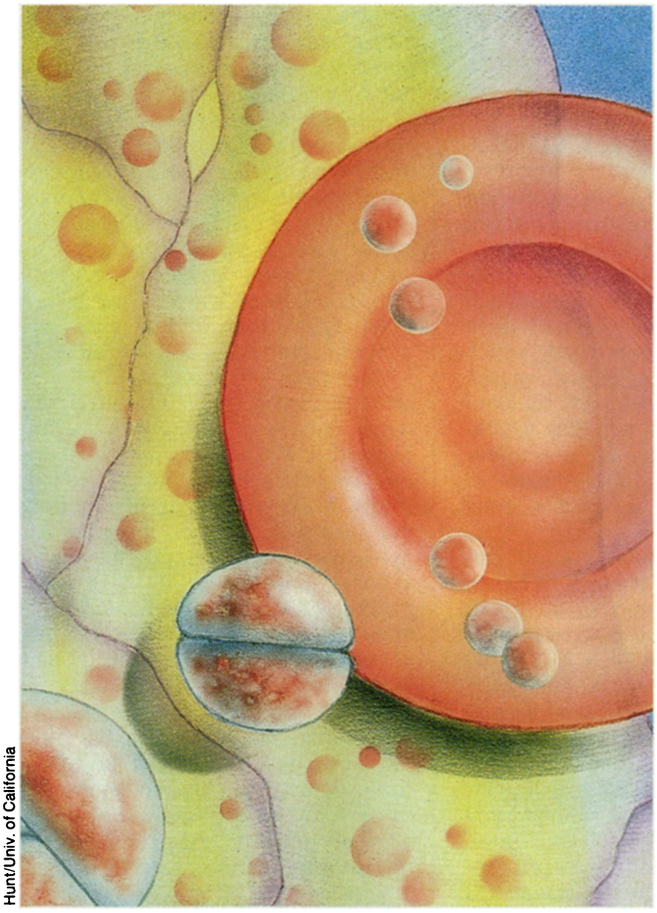
The substance is blood, and in every culture, in every time, it has been universally associated with life itself.

But today that view is beginning to change, as a growing number of blood recipients find themselves at risk of contracting a variety of infectious diseases. And while the chances of being transfused with AIDS-contaminated blood are now estimated by federal officials to be less than one in 50,000, other complications are not so unlikely. Non-A, non-B hepatitis, for example, is a potentially deadly disease that now afflicts one of 10 blood recipients.

Such risks have left many people afraid to receive — or even give — what the American Red Cross has called "the gift of life," and has spurred renewed interest in an old biotechnical quest to develop a marketable "artificial blood."

Artificial blood has circulated for years in the veins of science fiction's semi-synthetic characters. But real-live biomedical scientists have also been experimenting — in some cases on human patients — with several varieties of high-tech crimson.

Fear of infectious disease is not the only incentive. The military is interested in a battlefield-ready product with a longer shelf life than that of biological blood. And a type-free blood substitute would bypass many of the problems of finding compatible donors for recipients with unusual antibodies. Indeed, the Food and Drug Administration has for more than 20 years encouraged the development of artificial blood, with promises to expedite the cumbersome regulatory process if someone would just make a product that works. But no such product has yet been approved for anything other than experimental use — testimony to the biological complexity of the priceless red wine flowing through our veins.



Hunt/Univ. of California

View inside a capillary, in which blood has been replaced with a 25 percent suspension of hemoglobin-containing synthetic neohemocytes. A normal red blood cell is shown for scale. Unlike the doughnut-shaped living red blood cell, neohemocytes are nearly spherical and may have one, two or three chambers.

"Physiological systems always turn out to be more complicated than we thought," says C. Anthony Hunt, a blood-substitute researcher and professor of pharmaceutical chemistry at the University of California at San Francisco (UCSF). "Even red blood cells alone do so many things. Yet today's red blood cell substitutes are being designed to do just one of those things: transport oxygen to tissues."

In addition to red cells, he notes, there are white cells and platelets and a host of dissolved proteins in biological blood — far too complex a system to mimic in the lab. "Current biotechnology is capable of making single-protein copies, but isn't able to assemble things into complex systems," he says. "It's the difference between making a single piece of pasta and a finished fettucini alfredo."

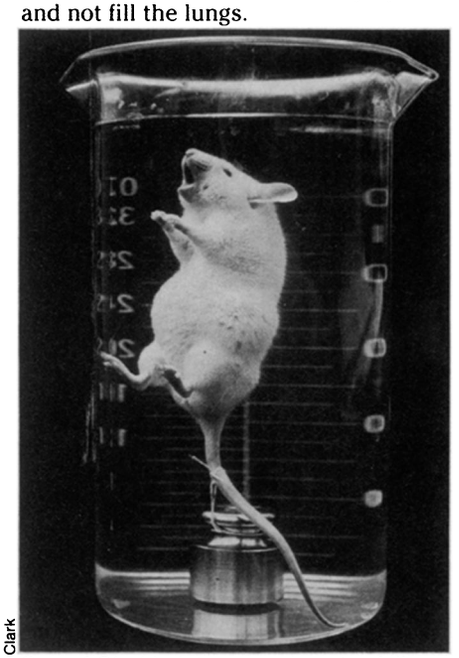
Still, he points out, progress has been made in the quest for the perfect blood substitute. He lowers his voice in a tone of near-reverence: "I'll never forget the picture of that rat, immersed and kicking around in a beaker of Fluosol. We were all so excited. It seemed like a panacea."

The rat that Hunt remembers belonged to Leland Clark — an "eccentric and brilliant" researcher at the University of Cincinnati, according to George Nemo, chief of the Blood Resources Branch of the National Heart, Lung, and Blood Institute (NHLBI). In 1965, Clark was experimenting with silicone oil as an oxygen carrier, in the hope that it might lead to the development of a red blood cell substitute.

To demonstrate the oxygen-providing potential of the oil, Clark performed a rather memorable experiment: He filled up a beaker with the synthetic fluid and dropped in one of his experimental rats. As with any drowning animal, the rat's lungs filled with the fluid and it sank to the bottom. But lo and behold, it went on breathing nevertheless.

"The work really began when I wondered whether or not a rat could breathe silicone oil, since silicone oil dissolves about as much oxygen in a given volume as there is oxygen in air, namely 20 percent," Clark recalls. "I knew that silicone oil was unique and biologically inert, so I simply put a rat in silicone oil after bubbling the oil with oxygen, and much to everybody's surprise he breathed it and stayed pink and lived."

Silicone oil is less dense than water, so the rat sank to the bottom very quickly, Clark says. "The first ones stayed down about 10 minutes; then we pulled them out and they were drained and they lived for two or three days." Subsequent lung inflammation may have caused the rats to die, Clark says. He notes that under normal conditions a blood substitute would remain in the circulatory system and not fill the lungs.



Clark

One of Clark's later experiments: A live mouse "breathes" oxygen in a high-density fluorocarbon liquid.

Clark's startling experiments inspired other researchers to test related fluorocarbon liquids for their usefulness as blood substitutes. That led to the development of a product called Fluosol-DA, a synthetic, acellular oxygen carrier made from two fluorine-containing (perfluorocarbon) compounds and — among other things — an egg yolk constituent and a mild detergent.

"For a while there it was the front runner," says Nemo, of NHBLI, whose grants have supported some of the Fluosol research. "It was the only one to make it to human clinical trials, but it had a few problems."

The problems were daunting. In one crucial trial at the University of Chicago Medical School, Fluosol was transfused into 23 patients suffering from acute anemia who had refused blood transfusions on religious grounds. The researchers concluded that Fluosol-DA was "unnecessary in moderate anemia and ineffective in severe anemia."

"It's hard to prove [Fluosol's] efficacy," concedes George Groveman, director of new products marketing at Los Angeles-based Alpha Therapeutic, a subsidiary of Japan's Green Cross Corp., Fluosol's manufacturer. "But as a matter of fact. . .if you had to prove the efficacy of blood to the satisfaction of the Food and Drug Administration, you could not get blood on the market, period." And as far as safety is concerned, Groveman chides, alluding to problems with real blood, "We certainly didn't contaminate anybody with viruses."

For now, the company has turned its attention to other, more promising uses for Fluosol — as an "ischemic modifier" and a radiation therapy enhancer (see box).

Meanwhile, Hunt and his colleagues at UCSF have been perfecting a method of making "neohemocytes" — synthetic microcapsules

In a similar vein: Fluosol-DA

Perfluorochemicals are fluorine-containing compounds, many of which are able to carry large quantities of oxygen. Fluosol-DA, first developed in Japan, is a patented emulsion of two such perfluorochemical compounds, perfluorodecalin and perfluorotripropylamine. It is currently undergoing human clinical trials for a variety of potentially therapeutic uses. Among them:

• *As an "ischemic modifier" in conjunction with balloon angioplasty:* Balloon angioplasty, in which a tiny balloon is inserted and temporarily inflated inside a coronary artery, has become a popular method of unclogging blood vessels that are responsible for bringing oxygen to the heart. During the procedure itself, however, blood flow to the heart is blocked, limiting the time allowed for the delicate operation. Fluosol is being used experimentally in animals to oxygenate the heart during this induced "ischemia," or lack of oxygen.

It's hoped that the technique will allow physicians to leave the balloon inflated for longer periods, as longer inflation time is believed to make the vessel treatment more permanent.

"Fluosol has a very small particle size — much, much smaller than any red blood cell," says George Groveman, of Alpha Therapeutic, which holds the U.S. license to manufacture and test Fluosol. "A Fluosol particle is about one-seventieth the size of a red blood cell," which allows Fluosol to go "where the delivery of blood is either not possible or is submaximal."

• *As a cancer therapy enhancer:* Nearly all solid cancer tumors contain cells that are extremely low in oxygen — a property that makes them resistant to both radiation therapy and chemotherapy. "Both modes of therapy require high levels of intracellular oxygen to exert their maximum effect," Groveman says, "so by oxygenating a tumor with Fluosol we are able to sensitize it to radiation or to chemotherapy." Advanced clinical trials along these lines have been "very encouraging," he says.

• *As a preventive of reperfusion injury:* Due to a process called free-radical superoxidation, cells just behind a recently reopened clot are often injured with the first rush of oxygenated blood. But the damaging reaction is apparently catalyzed by white blood cells, and there is hope that an acellular oxygen carrier such as Fluosol may be useful as a reperfusion fluid immediately following clot-dissolving therapy.

• *As a cardioprotective agent during heart attack:* Animal trials suggest that Fluosol may protect heart muscle if administered during a heart attack. "We're still looking at what kind of 'cocktail' would be involved," Groveman says. "But the dog trials that we've done suggest that you can salvage a third to two-thirds of that area of heart muscle that would have been infarcted [injured by lack of oxygen during a heart attack]."

In addition to these uses, some researchers believe that Fluosol may prove useful for keeping donor organs oxygenated while they are readied for transplant. And it may be an effective treatment for cerebral ischemia, in which an injury results in an interruption of oxygen flow to certain parts of the brain.

A Fluosol-like oxygen carrier may even get approval as a systemic "hemoglobin substitute" for patients with seriously low levels of the natural oxygen carrier. But clinical trials are difficult to perform on such seriously ill patients, Groveman says. So for now, the research emphasis is on "localized, focal ischemic events, as opposed to a generalized lack of oxygen." As such, he says, "We expect it to be on the market sometime next year." — *R. Weiss*

that look like miniature biological cells. Inside each neohemocyte Hunt traps a quantity of hemoglobin, the oxygen-carrying substance found in normal red blood cells.

Hunt's microcapsules are modified versions of a product originally developed by 3M Corp. for making carbonless carbon paper and "scratch and sniff" ads. In those products, microcapsules are filled with either ink or a scent, which is released when the capsules are cracked by pressure or by scratching.

But while microcapsule technology has been around since the 1950s, scientists have had a difficult time adapting it for biological use. Part of the problem is that hemoglobin, in comparison to ink or perfume, is not very stable once it's been harvested from biological cells.

"For the past 20 years," says Hunt, "research labs have been bashing their heads against the walls to develop a biocompatible microcapsule that won't destroy hemoglobin."

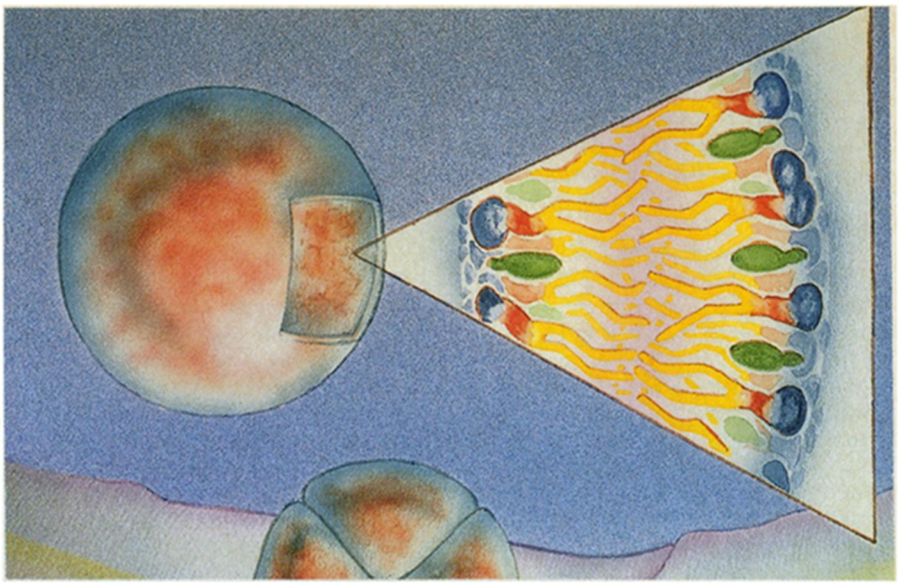
Hunt has found some success with his microcapsules, which are made, in part, from phosphorus-containing fats, cholesterol and an ingredient found in egg yolks. But while the hemoglobin appears willing to reside in such "cells," the body is not so easily fooled. Experiments with animals have shown that most of the artificial cells survive only a few hours before they are recognized as foreign intruders, broken down, and their pieces removed from circulation.

Such complications have led some researchers to forget about microcapsules altogether, and to experiment instead with free-flowing, chemically modified hemoglobin. One advantage is that hemoglobin by itself can circulate through the body without being noticed by the immune system. But there remain problems with this method as well.

First, hemoglobin molecules are very small, and are easily lost from the circulatory system if not encased in some kind of cell. Another problem is that without certain enzymes that are normally found in red blood cells, hemoglobin has a tendency to hang on to its oxygen rather than give it up to oxygen-starved tissues. So while a patient's chemically modified blood may be perfectly well oxygenated, the *body* may suffocate nevertheless.

Despite these obstacles, Enrico Bucci, a blood-substitute specialist at the University of Maryland School of Medicine in Baltimore, remains optimistic about chemically modified free-flowing hemoglobin.

"In my opinion, most of these problems have been solved, at least at the [laboratory] bench," he says. Bucci uses chemicals to link several hemoglobin molecules together, making them less easily lost from circulation. He has also been successful in using chemicals to modify the oxygen affinity of hemoglobin.



Hunt/Univ. of California

Cutaway view shows structural detail of a neohemocyte "cell membrane," made of two kinds of phospholipid and cholesterol. Oxygen can diffuse across the membrane from hemoglobin that is trapped inside.

Other problems persist, however. During the production process, for example, it has been difficult to keep the hemoglobin solutions free from bacterial endotoxins — potent poisons that have a penchant for clinging to hemoglobin. In addition, through a process known as superoxidation, hemoglobin has the potential to damage neighboring cells. And there is increasing evidence that free-flowing hemoglobin solutions induce a generalized constriction of the body's blood vessels, making oxygenation of tissues more difficult.

Says Hunt: "It's no accident that in nature hemoglobin is always found in a cell."

But perhaps the biggest problem with such hemoglobin-based blood substitutes is that the hemoglobin must be derived from blood itself. Biotechnologists are still a long way from being able to clone such a complicated protein. And recent reports suggest that even chemically modified hemoglobin-based substitutes still carry some risk of transmitting viral and bacterial infections.

"I think in a general way, whole-blood technology, or natural product technology, is going to be in competition with purely synthetic, clean, reproducible technology," says Clark, the researcher who dunked rats in silicone oil. Fluorocarbon fluids such as Fluosol can be made from "completely synthetic, controllable components," he says. "You'll know the exact structure of everything in it, and you don't have to hope that you get it all cleaned up. Endotoxins, all kinds of viruses, retroviruses, you name it — there's a long list of things that are already known to be in the blood pool, so you hate to start with such dirty stuff and always be nervous about it."

Moreover, he says, the ingredients for truly artificial blood are easy to come by. "You start from calcium fluoride, which is a mineral, extremely plentiful on earth, and some products derived from oil or coal, of which there's an unlimited supply relative to the quantities we're talking about."

From the FDA's point of view, however, modified hemoglobins may be farther along. "A lot of people [doing free-hemoglobin research] are saying that they're close to phase one clinical trials," says Thomas Zuck, chief of the FDA's Blood and Blood Products Division. "But lately I haven't heard anything — *anything* — about using perfluorocarbons as a circulating blood substitute."

Nemo, of NHLBI, is more optimistic. "There's still a lot of work to be done, but I think there may be some good compounds on the horizon." Specifically, he says, "There are some new second- and third-generation types of perfluorochemical compounds that still have to be tested in laboratory animals but which look very promising."

Clark concurs. "The first uses will be with specialized things like keeping hearts in better condition during catheterization, and it will be useful in the treatment of stroke and things like that." But it will not be long from now, Clark says, when perfluorocarbons will start to replace blood as a multipurpose circulating fluid.

"They say I'm an optimist; well, you have to be to do scientific work for very long. When somebody asks me how long it's going to be before we have artificial blood, and I say two years, my wife says, 'Well, you can tell everyone that you sure are consistent, because you've been saying that for 10 years now.' " □