chemical causes a particular disease. Furthermore, to ensure that laboratories produce reliable results, common standards and independent quality assessments are required. The European Union (EU) is therefore funding efforts to harmonize biological monitoring practices throughout the EU. Being wrong can be costly and carries serious risks for individuals and society.

What biomonitoring does yield is a far more accurate estimate of human exposure than do indirect methods. Furthermore, better knowledge of the distribution and metabolism of toxic chemicals in the human body, coupled with improved tools to make more accurate measurements, will further refine the technology. The next step in improving biomonitoring is to identify biological markers that would allow researchers to determine the effects of the interactions of chemicals in specific target organs or cells. "Eventually, we will be able to link exposures with early biological markers of effects," said Sexton.

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A bloodless revolution

A growing interest in artificial blood substitutes has resulted in new products that could soon improve transfusion medicine

Blood transfusion was once regarded as a safe and effective practice to save a patient's life after massive blood loss. But the AIDS epidemic and concerns that donated blood could be contaminated with HIV and other infectious agents, such as the hepatitis C virus, shattered public confidence in blood transfusions. This only added to already existing supply problems. In the USA alone, about 4 million patients require some 10–12 million blood units each year. About two-thirds of these are used to replace blood loss after trauma and surgery, and the remainder is used in chronic blood loss, cancer and anaemia. Because the demand for blood products continues to increase due to more sophisticated surgical procedures and the expanded health needs of an ageing population, there are often seasonal and regional shortages. The increasing demand and the loss of public faith in transfusion safety has revitalized the search for artificial blood substitutes. After many failed attempts, researchers in academia and industry are now ready to test the first promising products in laboratory and clinical trials. However, replacing our lifeblood is not as easy as it seems. "Development of an effective and safe oxygen carrier to be used instead of blood transfusion is an extreme challenge," said Robert Winslow, founder of Sangart (San Diego, CA, USA), a company that develops blood substitutes.

Although the idea of using a blood substitute in transfusions was first advanced in the seventeenth century by the British scientist Sir Christopher Wren, real efforts to develop

such a product only began in the early 1930s. William Amberson and colleagues at the State College of Medicine in Memphis (TN, USA) showed that bovine haemolysates could transport oxygen in mammals (Amberson *et al*, 1933), and later found that human haemolysates had the same potential when infused in patients (Amberson *et al*, 1949). Since then, the development of transfusion medicine has received much of its impetus from the military—not surprising, given that haemorrhage is the leading cause of death on the battlefield. In the 1960s, during the Vietnam War, the US Army began to collaborate with several private biomedical companies and supported research on blood substitutes based on different approaches.

Wartime experience guided developers in delineating the ideal properties of an oxygen carrier for military use: universal compatibility, and thus no time-consuming testing for blood type; no transmittable pathogens or allergens; long-term storage capability, preferably under non-refrigerated conditions; safety and non-toxicity; and superior oxygen delivery capability, resulting in oxygenation of peripheral tissues even after massive blood loss. Intuitively, this describes applications that go far beyond wounded soldiers, ranging from immediate treatment of patients in haemorrhagic shock after traumatic injuries, to relieving shortages and avoiding contaminated supplies, to assuring intervention even when religious objections or rare blood groups prevent normal blood transfusion. Additional uses can be envisaged in a variety of diseases with

THE OTHER SIDE OF THE MEDAL

Among the possible applications of haemoglobin-based oxygen carriers (HBOCs) as blood substitutes, there is one that would not make their inventors proud: blood doping in sports. Indeed, HBOCs are a real temptation for athletes who are willing to use illicit means to boost endurance and performance. International sporting federations are already taking this possibility seriously and have added HBOCs to their list of banned substances (Schumacher & Ashenden, 2004). But prohibition is only half the solution, and scientists have had to develop a test for the presence of artificial oxygen carriers. "A criticism often levelled at antidoping agencies is that they continually play 'catch up' with drug cheats, inasmuch as the athletes are continually finding new drugs to abuse before antidoping agencies have implemented detection methodologies," said Michael Ashenden, the project coordinator for the Science and Industry Against Blood Doping (SIAB; www.siab.ws) research consortium. "In the case of HBOCs, proactive research has prevented this from happening." Due to SIAB's efforts and the fact that pharmaceutical companies developing blood substitutes agreed to provide early access to their products, it was possible to have a generic test in place for HBOCs in time to prevent the infiltration of these products into the world of athletics. "Subsequently, athletes were deterred from abusing HBOCs because of the risk of being caught—and no positive test for HBOCs has been recorded in any sport," Ashenden said. The main challenge for the SIAB researchers was to develop a test capable of detecting all known blood substitutes at once, rather than requiring a separate test for each product (Schumacher & Ashenden, 2004). Because blood substitutes are not excreted in the urine, it was also necessary to develop detection methodologies that used blood as the test matrix. Finally, as HBOCs have a half-life in the circulation of only 12–24 hours, the test must be conducted soon after competition.

compromised tissue oxygenation, such as heart infarct and stroke, in tumour oxygenation, sickle-cell anaemia, organ preservation, autoimmune haemolysis or air embolism (Chang, 1997). And there is, of course, a strong interest among athletes who are willing to boost their performance by illicit means (see sidebar).

In the early stages of research, it was clear that it was not necessary to develop a substance that mimics all the functions of whole blood, but rather a temporary oxygen carrier to provide adequate O₂ perfusion and CO₂ removal under physiological conditions until the patient's bone marrow has produced sufficient new blood cells. Another key requirement for resuscitation of patients in shock is the restoration of circulatory plasma volume (Moore *et al*, 2004), so the ideal blood substitute should provide both oxygen transport and volume expansion.

The first obvious candidate was haemoglobin (Hb), the oxygen-carrying molecule itself, but Amberson had already noted serious complications with this approach, including vasoconstriction, abdominal pain and acute kidney failure (Amberson *et al*, 1949). Amberson attributed these toxic effects to contaminants of Hb solutions, but toxicity persisted even when highly purified Hb became available. Indeed, Hb is toxic *per se*, because outside a red blood cell, its tetrameric structure is rapidly broken down into dimers and monomers that are taken up by the kidney where they impair nephrological functions. Furthermore,

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because of the absence of 2,3-diphosphoglycerate (2,3-DPG; a compound in erythrocytes that binds Hb and decreases its affinity for oxygen), free Hb lacks the delicate oxygen binding–releasing equilibrium and tends to keep O₂ bound at the expense of peripheral tissues. Another problem with cell-free Hb is its pro-oxidant activity—due to uncontrolled haem-mediated oxidative reactions and interactions with various oxidant/antioxidant and cell-signalling systems—which can aggravate oxidative stress (Alayash, 2004). Hb also drives the amplification of systemic inflammatory responses by monocytes (Simoni *et al*, 2000). "Red blood cells not only carry Hb and protect it from the body's proteolytic enzymes, but also the body from Hb's oxidative toxicity," commented Abdu Alayash at the US Food and Drug Administration (FDA) Center for Biologics Evaluation and Research (Rockville, MD, USA).

To complicate the picture, Hb can enter the interstitial spaces between the endothelial cells that form the walls of blood vessels, where it scavenges nitric oxide (NO) and thus induces vasoconstriction and hypertension. This could be beneficial for trauma

patients suffering from severe blood loss, but also has other life-threatening consequences (Olson *et al*, 2004). "Protein engineering has been employed to counteract this problem, for instance by modifying the active site to reduce the rate of combination of NO with deoxy- or oxyhaemoglobin," said Maurizio Brunori, a leading haemoglobin biochemist at the University of Rome 'La Sapienza' (Italy). "We have also introduced multiple modifications of the distal haem pocket in order to control NO reactivity and thereby reduce this adverse hypertensive effect." However, not everyone agrees that Hb-mediated NO scavenging is the only mediator of vasoconstriction. Indeed, the process might be more complicated and could involve the regulation of vascular tone by oxygen itself (Vandegriff *et al*, 2004). "It seems obvious that there is more than one mechanism responsible for vasoconstriction," commented Brunori, "and certainly reducing accessibility of the modified haemoglobin to the immediate environment of the cells of the endothelium [where NO is synthesized] can avoid or at least reduce problems related to NO scavenging."

Given these problems with pure Hb, two main strategies for the development of blood substitutes have emerged: oxygen carriers that are based on modified Hb; and perfluorocarbon (PFC)-based products. Much of the research so far has concentrated on the first approach, as Hb can be obtained easily in sufficient quantity from animals—mainly cattle—or from outdated human blood, or can be produced transgenically, so the raw material is not limiting. The real challenge is how to control the negative side effects of the protein. Various researchers have therefore tried to stabilize the Hb tetramer by using recombinant techniques or chemical crosslinkers and have added 2,3-DPG analogues to reconstitute the normal oxygen affinity of Hb.

Crosslinking indeed prolongs the circulation time of Hb and eliminates kidney dysfunction and other undesirable effects, except vasoconstriction. This is because stabilized tetrameric Hb is still small enough to slip in between the endothelial cells of vessel walls and bind NO. The next logical step was to extend the crosslinking concept to obtain large polyhaemoglobins of up to several thousand Hb molecules linked by intramolecular and intermolecular bridges. Apart from

having limited or non-existent vasoconstrictive activity, these polymers exert less colloid osmotic pressure than free Hb and can therefore be administered at higher doses.

This now provides the basis of several Hb-based blood substitutes under development. PolyHeme®, a glutaraldehyde-polymerized human Hb developed by Northfield Laboratories (Evanston, IL, USA), is now being tested in a phase III trial for early treatment of trauma patients. "If the trial meets its endpoints, and we are confident that it will, PolyHeme has the potential to improve survival in critically injured patients, whether on the streets or on the battlefield," commented Steven Gould, Northfield's Chairman and CEO. "It could transform the treatment of trauma in the United States and throughout the world."

Sangart focuses on Hb-based oxygen carriers from a different perspective. The company's main product, Hemospan™, is a non-polymerized human Hb molecule with its surface modified by polyethylene glycol (PEG). PEGylation eliminates the toxicity of free Hb, increases its effective size thus prolonging circulation time, suppresses vasoconstriction and provides a viscosity that is similar to natural blood (Winslow, 2005). "Hemospan is a representative of a new class of oxygen carriers," said Winslow. "It is the only product, of which we are aware, that embodies the new understanding of the microcirculation and its control." According to Winslow, Hemospan—which is anticipated to enter phase III trials early next year, combines low Hb concentration with high oxygen transport capability, to minimize any potential side effects. It is also easier to produce. "A significant advantage of Hemospan over competing products is that its cost of manufacture is relatively low and the yield from raw haemoglobin is very high," said Winslow.

However, faith in this type of product is not universal. "Negative results in preclinical studies should be carefully analysed before Hb-based oxygen-carrier solutions are used in large clinical studies to avoid harm to the patients," warned Hans-Georg Bone and Martin Westphal from the University of Münster, Germany, in an editorial discussing the mixed outcomes of preclinical and clinical trials of different blood substitutes (Bone &

...it was clear that it was not necessary to develop a substance that mimics all the functions of whole blood, but rather a temporary oxygen carrier to provide adequate O₂ perfusion...

Westphal, 2005). Furthermore, avoiding side effects might require a drastic refinement of basic strategies. "None of the current manufacturers are using recombinant DNA technology to engineer more efficient and safer haemoglobin products," said John Olson from Rice University (Houston, TX, USA). Olson firmly believes that mutant Hb could address not only the hypertensive effect, but also other crucial

issues such as oxygen affinity and shelf life, with the extra advantage that "supply of recombinant haemoglobin for oxygen carrier production is potentially unlimited because the material is manufactured in bioreactors from simple media and not dependent on human or animal donors." Regardless of the outcome, "in contrast to the prospects [a few years ago]...there is still a long stony road ahead," Bone and Westphal wrote, at least for 'traditional' Hb-based blood substitutes, and recombinant Hb is still too expensive to be competitive with the first-generation products, said Olson.

Thomas Chang, a pioneer in blood substitute research at McGill University (Montreal, Canada), is pursuing an even more revolutionary vision: artificial red

blood cells (Chang, 2005). He has already produced a second generation of Hb-based oxygen carriers formed by linking polyhaemoglobin with the antioxidant enzymes superoxide dismutase and catalase. In animal tests, this combination prevented the ischaemia-reperfusion injuries caused by oxygen radicals that can be produced by reperfusion with an oxygen carrier alone (Chang, 2005). There are also "third generation and more complete red blood cell substitutes [in development] with longer circulation time in the form of haemoglobin lipid vesicles and... biodegradable polymeric membrane nano-artificial red blood cells with the same content as red blood cells," said Chang.

The main alternative to Hb-based solutions are PFCs, which are hydrocarbon-like substances with fluorine instead of hydrogen atoms. In the 1960s, these compounds were found to be capable of dissolving 50% or more of their own volume of O₂—about 100 times more than plasma—and large amounts of CO₂. Unlike Hb, gas molecules are not chemically bound to PFCs, but are absorbed and released by simple diffusion. An O₂-saturated PFC solution injected into the bloodstream could thus easily replace erythrocytes without the common side effects of Hb, but PFCs have other drawbacks. They are insoluble in water and must be emulsified before infusion, and they are rapidly removed from circulation and sequestered in the reticulo-endothelial system, where they can cause complications. Furthermore, the oxygen-carrying capacity of PFCs depends on the oxygen partial pressure to which the solution is exposed, which also limits their use to situations with supplemental oxygen and controlled ventilation.

Substituting blood is a complex process and even the latest products may need further refinement...

Despite these hurdles, the only oxygen carrier approved so far by the FDA is a PFC emulsion named Fluosol®, developed by Green Cross Corporation in Osaka, Japan. Until the FDA rescinded its approval in 1993, surgeons used it for enhancing oxygenation of the heart during coronary artery balloon angioplasty.

Some believe that the various benefits of PFCs, such as their easy and cheap production and prolonged storage capacity, make them worth exploring further. Alliance Pharmaceutical Corporation, a biomedical company based in San Diego (CA, USA), is now developing a new PFC emulsion, Oxygent™, designed to overcome the problems that eventually caused the FDA to pull Fluosol from the market. Oxygent is intended to reduce the need for donor blood during surgery; it has already completed a phase III clinical study, the results of which showed a significant reduction in the need for transfusions compared with controls.

Whether the Hb- or PFC-based products under trial are eventually approved for clinical or battlefield use remains to be seen. Substituting blood is a complex process and even the latest products may need further refinement to overcome various side effects. "Each new generation of red blood cell substitutes is increasingly more complicated and expensive," said Chang about his research on artificial erythrocytes, but his comment applies to other approaches. Nevertheless, given the great demand for artificial blood substitutes and the increasing interest from academic researchers and biomedical companies, he and others are positive that future products will be safe enough for widespread use in surgical theatres and in the field, to rescue people after massive trauma.

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A nutraceutical a day may keep the doctor away

Consumers are turning increasingly to food supplements to improve well-being when pharmaceuticals fail

The atmosphere at the seminar on 'Treating and Preventing Chronic Illness with Natural Asian Therapies' was part medical conference, part pilgrimage to Lourdes. About 250 elderly and

health-conscious adults crowded into the conference room of an upscale Manhattan hotel in May, listened eagerly, and asked urgent questions after researchers from Columbia University's Center for Holistic