

ARTIFICIAL BLOOD: A BEGINNER'S GUIDE

Ever since William Harvey's description that blood was a circulating liquid in the 17th Century, scientists have been trying to replace blood with other liquids. Originally it was thought this might cure diseases and change personalities. Wine and milk were amongst the earliest favoured materials, with interesting, if perhaps not wholly useful side-effects.

Nowadays we replace blood only when it has been lost due to some trauma or in severe anaemia. And we replace it with blood from a suitable donor. In most countries giving blood is a voluntary unpaid task. It is one of the more altruistic deeds we humans perform - at a moderate cost to ourselves we provide a resource that will help a fellow person in a critical moment of need. And what could be more precious than donating one's lifeblood?

Of course, as the evolutionary biologist will tell us, most examples of apparent altruism can readily be seen as an evolved behaviour that will assist in the survival of our genes. If no one gave blood then we would have no blood when we needed a transfusion; a case of I scratch your back and you scratch mine (or reciprocal altruism as the jargon would have it).

However, only 90% of the blood donated is actually transfused into another person. This means that the blood from over 1 million donations in the USA per year is not used. Although this might seem a terrible waste it is not quite so bad as it seems. Many medical products can still be made from "outdated" blood as well as the blood providing raw materials for medical research.

Still the fact that we can't use all that blood "efficiently" is one of the reasons why many pharmaceutical companies have spent millions of dollars and billions of yen in order to develop artificial replacements for blood transfusions. Blood is a complex solution with many different products; some of these are not stable and may degrade making them less useful, or worse still, toxic. Its "shelf life" in the fridge is 40 days. Longer than your milk, but not as long as your cheese.

This short shelf life would be less of a problem if blood were blood the world over. Then at least you could always use the blood closest to its "use before date" first. However, each person's blood is not the same; unfortunately different people have different blood groups (O, A, AB, B are the major ones, but many minor sub-types and variations exist). These represent molecules on the surface of blood cells. If you are given blood that is from the wrong group, then your body recognises the alien molecules and attacks them, with potentially fatal consequences.

So we need to store not just one type of blood, but many. Typically 8 different types are available at any one time in the average hospital. Now our storage system has to be much more complex. We will not always be using the blood closest to its "use before date" as it will be of the wrong type. Therefore some blood will end up being wasted.

One possibility is to convert blood groups from one to another; amazingly it has been demonstrated that mixing blood with coffee beans can change type B blood into type O. However, the long-term aim is to develop a product that can be stored safely for long periods. Ideally the product would be in a powder form for rehydration with a salt solution when desired. What we are looking for is the powdered milk equivalent for blood. The blood could be stored in a packet and rehydrated when required. Such a product would also not need to be stored in a hospital. Paramedics could carry it to an accident and start a "blood" transfusion on the spot. Furthermore if the "blood" were free of cells as well, then there would be no problems with blood group matching. In the light of this it is perhaps not surprising that one of the major players developing these artificial blood products has been the US army.

However, the above factors represent a problem that would be nice to solve, but not essential. With the exception of groups such as Jehovah's Witnesses (who refuse to accept blood transfusions) the system by and large works at present - or does it? In fact the major factor currently driving the research and development of artificial blood substitutes is not convenience and shelf life, but sterility. The AIDS epidemic shook the transfusion industry when it was realised that a new lethal blood borne virus (BBV) could be transmitted via donation of blood and blood products. Equally dangerous, but less well known, is Hepatitis, which can be caught in a similar way. At least with Hepatitis there are vaccines against some of the strains; AIDS vaccination is still many years off.

We can now test for, and discard if necessary, blood that contains HIV, the virus that causes AIDS. Prior to the development of tests many thousands of people were infected with HIV via blood

transfusions from infected donors. A similar number of haemophiliacs were infected from blood clotting factors that contained the virus. The problem is that all the treatments that can kill these viruses render the blood useless for donation, as they damage other parts of the blood. The problem for the blood transfusion service is that they can only test for the viruses they know about. If a new virus appears as swiftly as HIV, then blood will be a dangerous product once more (until the new virus is characterised and a test for its presence can be developed). The vital importance of blood transfusion as a therapy, coupled with the long-term doubts over the safety of current blood products, gives me confidence that in the next 10 years there will be a significant number of artificial blood substitutes on the market that are used instead of blood in some, or perhaps all, circumstances.

So what are the requirements for developing a good blood substitute? Our metabolic fuels are primarily fat and carbohydrates (sugars). The most efficient way to get energy from these fuels is to react them with molecular oxygen. Therefore the more oxygen we can carry around the body the more efficiently we can get energy from burning our sugar and fat fuels in that oxygen (a process called cellular respiration). At the level of the elite athlete more dissolved oxygen means an ability to run faster at "aerobic" events. For the track athlete oxygen delivery is important for any event longer than 300 meters long, but it is in the long distance events where extra oxygen carrying capacity is most critical - hence the hunt for legal and illegal activities designed to increase the amount of oxygen that can be carried around the body and delivered to the muscles; these include high altitude training, blood boosting and erythropoietin doping (the cause celebre brought to the media's attention by the Tour de France scandals in cycling). At the other extreme if we lose a lot of blood then there comes a point where we can no longer deliver enough oxygen around the body to survive when lying down, let alone when attempting to run a marathon. It is at this stage that we need more oxygen delivered around our body fast, and a blood transfusion is required.

Does this "oxygen" transfusion have to be blood? To understand the problems of making artificial blood that carries oxygen, we first need to understand how normal blood works. haemoglobin works.

Blood is made up of red blood cells that contain haemoglobin, a few white blood cells, platelets and plasma. The latter transports many useful molecules around the body e.g. blood sugar and fat. However, plasma is basically a sweet and salty solution of water. As anyone who has tried to hold their breath in the ocean or in a cup of sweet tea can attest to, although these watery solutions have many wondrous properties for life, oxygen transport is not one of them. This is because oxygen is not very soluble in water. What does the term soluble mean? Essentially the more soluble a molecule is, the larger the number of molecules that can be dissolved in a liquid. So if you add two spoons of sugar to your tea it is completely soluble, but if you try and add 200 you will find that there is a lump of undissolved sugar at the bottom of your cup. The tea has become saturated with sugar.

Sugar is a solid. However gases, such as oxygen, also dissolve in solutions. But oxygen does not dissolve well in the watery plasma. Fortunately red blood cells contain haemoglobin – the protein that gives blood its red colour. Haemoglobin allows more oxygen to dissolve in the blood by directly combining with the oxygen molecules (in the process of binding oxygen the haemoglobin changes colour which explains why your oxygen-rich arteries look red and your oxygen-poor veins look blue). For every haemoglobin molecule present, up to four extra oxygen molecules can be dissolved in the blood. This ensures that the vast majority (98%) of oxygen molecules in blood are transported bound to haemoglobin, not freely dissolved in the plasma. A number of drug companies are making artificial haemoglobin molecules to replace the oxygen carrying ability of red blood cells. These are pathogen free, do not need blood typing and have an extended shelf life. We shall return to the recent success of this approach later.

However, we can also increase the amount of oxygen in blood without using haemoglobin, merely by changing the solubility of oxygen in the plasma. To understand how this is done we need first to understand how and why gases dissolve in solutions. Let's start with some simple examples of dissolving a gas in a liquid. We don't usually encounter problems with varying oxygen solubility in our daily lives, but we certainly do with the another gas, carbon dioxide. Dissolved carbon dioxide is what puts the fizz in fizzy drinks. The carbon dioxide is forced into the drink under pressure to "supersaturate" the solution. This puts more carbon dioxide in the solution than would normally be present, but as the can is sealed (gas cannot get in or out) the carbon dioxide cannot escape. The best way to demonstrate this is with the famous beer can version of Russian roulette. Here a six pack of

beer is taken from the fridge and one can vigorously shaken. A "player" picks a can at random, holds it to his ear, and then pulls the cap.

Cheap lager (definitely the best sort for the above game) and coke use the high pressure method to get the gas into the can. Before I get any complaints from purists, there are of course other ways of putting gas into solutions that do not use high pressure. In these cases the can/bottle is sealed and a living organism forced to produce the gas. The humble yeast will happily ferment sugar into alcohol and produce carbon dioxide as a by-product. This is how you get the fizz into champagne or real ale.

There is an old wives' tale that says that if you do not finish your bottle of champagne then if you put the bottle back in the fridge with a fork dangling in it then the fizz will remain till the next day. I'd previously never tried this (champagne not remaining unfinished in my household) but in the spirit of scientific inquiry I did manage not to drink some champagne for this article. I can testify, via extensive and arduous testing that it is the low temperature of the fridge that keeps the carbon dioxide in the solution, not the act of putting a fork in the bottle. Remember that the concentration of gas in the champagne is artificially high and it will tend to escape to the air unless measures are taken. Gases are generally more soluble in cold solutions than warm ones and therefore more carbon dioxide is retained in the champagne left at fridge temperature than in that at room temperature.

Just like carbon dioxide, oxygen is less soluble at high temperatures. This has important implications for some organisms. Fish for example have a problem when the water temperature is raised. Raising the temperature increases their metabolism as most chemical reactions in their body speed up. Therefore they need to consume more oxygen; however, as we have seen due to the increase in temperature there is now less oxygen in the water they are breathing. Even more worrying is that all the other organisms in the fish pond require more oxygen as well. So the fish are in trouble and any fish that can develop a response to this lower oxygen in the water will be at an evolutionary advantage. This is an extreme problem in the hot waters of the Amazon river leading to many fish species develop lung-like organs enabling them to breathe air as well as water in such environments.

The reason we can't breathe underwater, but fish can, also relates to the low solubility of oxygen in water. Our metabolism (and hence requirement for oxygen) is much higher than the fish and we need the much higher amount of oxygen present in air than in water. At 20°C air is 20% oxygen and water is 0.001% oxygen. However, as we shall see later there is a way for us to breathe "underwater" and this will have important implications for the development of blood substitutes.

But we are getting ahead of ourselves. Although very nice in principle, it is clearly impractical to increase the amount of oxygen to a trauma patient by dropping their blood temperature (and anyway the gain in oxygen content is too small). Instead we need to take advantage of another old wives' tale. To show that I am not biased against old wives this one is true. Oil and water do not mix. Of course anyone who has made a French Dressing will know that you can temporarily defy the laws of physics (in this case thermodynamics) by vigorous shaking. But the oil and vinegar (which is largely water) will soon settle down again into different layers. Of course no laws are really broken as you have provided the energy to temporarily mix the two solutions, but what is it about water that the oil does not like? Well, water is a molecule made up of two hydrogen atoms and one oxygen atom (hence H₂O). Molecules contain small particles called electrons that can give them a negative charge. Now oxygen has a greater ability to hold onto its electrons than hydrogen. This results in the oxygen grabbing some of the negative charge from the hydrogen atom in the water. As a result of this molecular tug-of-war the oxygen end of the water molecule has a small negative charge and the hydrogen end a small positive charge.

It is a general physical principle that opposites attract (or is this an old wives' tale, now I'm getting confused!). Anyway the positive hydrogen from one water molecule is attracted to the negative oxygen from another molecule to form a network of molecules linked together. The technical term for these bonds (and for once the jargon relates to the process) is hydrogen bonds. Adding any uncharged molecules to this system would be bound to disrupt this structure and leave some of the positive hydrogens unbonded to the negative oxygens. Oil, whether olive, groundnut or rape seed, consists of large numbers of uncharged molecules. Dissolving them in water would create a structure around the oil molecule with unneutralised charges on the water molecules. This is energetically unfavourable so very little of the oil enters the water solution - result low solubility - unless you overcome this temporarily with a vigorous shake.

Oxygen gas consists of two atoms of oxygen bonded (stuck) together. However, with no positive-loving hydrogen around, unlike in the water molecule, the result is that neither of these atoms wins the tug-of-war and the oxygen gas molecule remains stubbornly neutral. This results in a similar problem to that of the oil - and is why oxygen is not very soluble in water. What is needed is a solution that does not contain these structured hydrogen bonds and in which oxygen can dissolve without requiring an input of external energy. By changing the molecular properties of the solvent you can increase dramatically the amount of oxygen dissolved in the solution. Fluorocarbons are neutral molecules that do not contain any hydrogen bond structure to disrupt and are relatively non-toxic. The solubility of oxygen in fluorocarbons is more than double that of plasma. By changing the physical properties of the plasma by replacing the water with molecules such as fluorocarbons we can increase dramatically the amount of oxygen dissolved in the blood. The problem of an artificial oxygen delivery system is thus essentially solved. The most dramatic demonstration of the increased oxygen content of a solution of fluorocarbon over that of water is that when a mouse is dropped into the milky white fluorocarbon solution it can breathe as easily as if it was in air. Anyone who has seen the US version of the science fiction movie "The Abyss" will have seen an example of this (the scene was censored in the more rodent-friendly UK).

At scientific conferences I have attended where blood substitutes have been discussed the community is divided into two opposing camps - those working on fluorocarbons and those working on artificial haemoglobins. Both products have had their day in the sun. The very first blood substitute was a fluorocarbon - Fluosol-DA – and was licensed for use in 1989. Then in 2001 a haemoglobin-based product (Hemopure) was licensed for use in South Africa. Hemopure is based on haemoglobin purified from cows' blood (naturally the herds are kept well away from the BSE-rich UK!). However, both products were removed from the market due to a combination of technical difficulties and concerns about possible toxic side effects. Current research aims to address these issues; there are even groups trying to make "real" red blood cells using stem cell technology. So sooner than you think you might be faced with the offer of a pint of the red stuff (haemoglobin) or the milky stuff (fluorocarbon) rather than good old blood donated from an altruistic citizen.

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