D(+)-Lactose and Other Sugars in Organ Preservation and Cryonics

By Mike Darwin

D(+) lactose monohydrate is the principal sugar in mammalian milks. The monohydrate part is easiest to explain; it simply means that the lactose molecule has one water molecule attached to it. This is important because some chemicals can have a lot of water molecules attached to them. For instance, you can have magnesium chloride with two attached water molecules (dihydrate) or six attached water molecules (pentahydrate). This becomes very important when you are weighing out a chemical and you need the chemical to be present in the correct amount. You'll understand how important this is if you consider that someone proposes to sell you a kilo of some very valuable chemical (say 100 times more valuable than gold per milligram). There is going to be a considerable difference in the amount (by weight and usually by volume) of the actual active chemical you get per milligram or gram (weight) depending upon how hydrated it is (how many water molecules it has attached. The molecular weight (molecular mass) of magnesium chloride is 203.30 and the molecular weight (MW) of water is 18.01. Now, if you have 6 water molecules for each magnesium chloride molecule you have a total mass of water of (18.01 x 6) = 108.06. That means if you have the pentahydrate salt of magnesium chloride you must add the weight of the 6 water molecules to the MW of magnesium chloride: 203.30 + 108.06 = 311.36. So, if someone is selling you a gram of magnesium chloride pentahydrate at the same price you can buy magnesium chloride anhydrous (no water) you are getting cheated because you are paying the same price for a gram of product that is 1/3rd water!

In biology and chemistry the same principle applies because if you need a certain amount of a chemical for critical reasons, say to maintain normal cell function or inhibit cell swelling in hypothermia, then you must account for any water molecules that may be attached to the chemical. In the case of magnesium chloride pentahydrate versus anhydrous magnesium chloride you are going to have to weigh out about 1/3rd more of the powder of the pentahydrate salt in order to get the same amount of magnesium chloride present in one gram of the anhydrous salt.

Why have pentahydrate, monohydrates, dihydrates and so on of chemicals? The answer is that some chemicals are almost impossible to handle in room air without rapidly absorbing water. Some chemicals will absorb just so much water and no more and thus are very stable under conditions of normal use, so they are supplied in this form. Some chemicals, especially organic chemicals, are virtually impossible to economically produce without one or more attached water molecules. Magnesium chloride is a really good example because it is intensely hygroscopic; it will literally pull water out of the air right before your eyes. So, not only is anhydrous magnesium chloride more expensive than the pentahydrate, it is virtually impossible to handle. If you try to weigh it out it will literally be grabbing water from the ambient air so fast that you can't tare it on the scale. In seconds you will see tiny droplets of water on the weighing boat or paper where the magnesium chloride has literally pulled so much water out of the air it is fully dissolved in a tiny droplet of water! Calcium chloride is just about as bad, so, you'll notice that we don't even bother trying to weigh these chemicals out as dry powders, but rather buy them as pharmaceutical

products for injection because they are already dissolved in solution in very precise concentrations:

Thus, it is much simpler to draw up the correct volume of these salts dissolved in solution to add the desired amount of these two chemicals to perfusate. It is possible to weigh them, but you have to be quick and it helps if you live the desert where the humidity is very low.

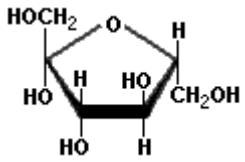
Now we come to the D(+) part which is much harder to explain. In the early part of the 19th Century the French physicist Dominique F.J. Arago noticed that when he passed polarized light through quartz crystals the light could be rotated either to the left or right depending upon the individual crystal. Shortly thereafter the brilliant physicist and mathematician Jean Baptise Biot (the Biot number, a dimensionless number used in unsteady-state (or transient) heat transfer calculations, is named after him) also observed this same effect in liquids and gases of organic substances such as turpentine and some other petroleum products. About 10 years later the English astronomer Sir Joun F.W. Herschel discovered that different crystal forms of quartz rotated the linear polarization in different directions. Simple polarimeters have been used since this time to measure the concentrations of monosaccharide sugars, such as glucose, in solution. In fact, one name for glucose, -dextrose-, is so named because it causes linearly polarized light to rotate to the right or “dexter” side. Similarly, levulose, more commonly known as fructose (fruit sugar) causes the plane of polarization to rotate to the left. Fructose is even more strongly levorotatory than glucose is dextrorotatory. Invert sugar which is formed by adding fructose to a solution of glucose, gets its name from the fact that subsequent structural conversion causes the direction of rotation to "invert" from right to left.

The reason for this behaviour of these seemingly identical substances was not understood until the mid-19th Century when Pasteur was working on the problem of why wine was souring as opposed to fermenting into an alcohol solution. The culprit was yeast that metabolized the fructose in the grape juice to tartaric acid. A solution

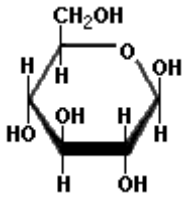
of tartaric acid derived from living things (the wine lees yeasts) rotated the plane of polarization of light passing through it, whereas chemically synthesized tartaric acid prepared by non-organic means in the laboratory did not have this effect. This was puzzling because both the synthetic and the biologically derived tartaric acid undergo the same chemical reactions and are identical in their elemental (atomic) composition. Pasteur noticed that the crystals came in two asymmetric forms that were mirror images of one another. He meticulously sorted the crystals by hand and then dissolved each of the two forms of crystals in water; solutions of one form rotated polarized light clockwise, while the other form rotated light counter-clockwise. An equal mix of the two had no polarizing effect on light. Pasteur deduced the molecule tartaric acid molecule was asymmetric and could exist in two different forms that resemble one another; as would left- and right-hand gloves, and that the organic form of the compound consisted purely of the one type.

This phenomenon is referred to as isomerism and occurs when two molecules have the same molecular formula (atomic composition) yet have different structures and therefore different chemical and physical properties. There are many different kinds of isomers. The two major divisions of isomers are the geometric and the structural. Structural isomers are isomers that have the same number of atoms but different arrangement of atoms. One structural isomer of glucose is fructose.

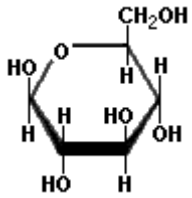
Fructose:



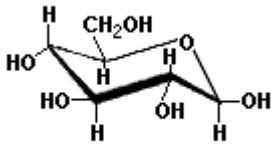
Various isomers of glucose:



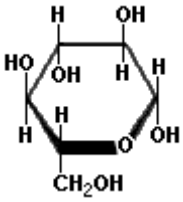
β-D-Glucose



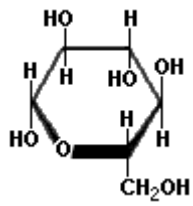
β-L-Glucose



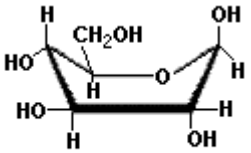
β-D-Glucose
(chair form)



β-D-Glucose



β-L-Glucose



β-D-Glucose
(boat form)

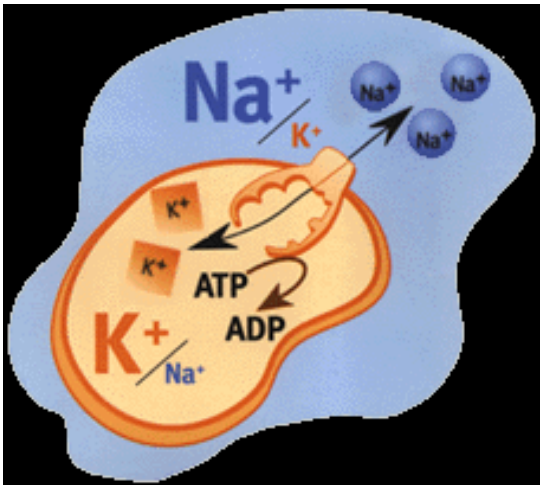
Geometric isomers are identical in arrangement of covalent bonds but are different in the order that the groups are arranged.

A major category is stereoisomers which are two isomers that have the number of atoms in the same order. A stereoisomer of glucose is galactose. In the Fischer projection all of the atoms are the same except for one rotated group. There are two categories of stereoisomers, enantiomers and diastereomers. Enantiomers are two isomers that are mirror images of each other when looked at in 3D while diastereomers are not. Galactose is just one of many diastereomers of glucose. To find out the possible number of stereoisomer forms a monosaccharide can have, you can use the formula 2x where x is the number of chiral carbons the molecule has. Molecular chirality occurs when a sugar has a carbon with four different groups attached to it. Any carbon with a double bond on it is never chiral nor are the end carbons. Because glucose has four chiral carbons there are 24 different stereoisomers; which means that there are sixteen different stereoisomers for glucose.

Two of the main divisions of glucose's many forms are l-glucose and d-glucose. These two are enantiomers which are determined by whether the two molecules are symmetrical at the last chiral carbon. When the hydroxyl group is on the last chiral carbon on the right it is considered d-glucose and when it is on the left it is classified as l-glucose. The "d" means that the glucose rotates polarized light to the right (dextrorotatory) and the "l" stands for levorotary (rotates polarized light to the left). These refer to how a plane of light rotates as it passes through a solution of it. First light is passed through a polarizing filter, then a polarimeter containing a solution made with the molecule. When a d-solution is in the polarimeter it will cause the light to turn to the right or at positive angle, while an l-solution will cause the light to turn to the left or a negative angle. Both d-glucose and l-glucose exist naturally but d-glucose, also called dextrose, is the most abundant sugar on the planet.

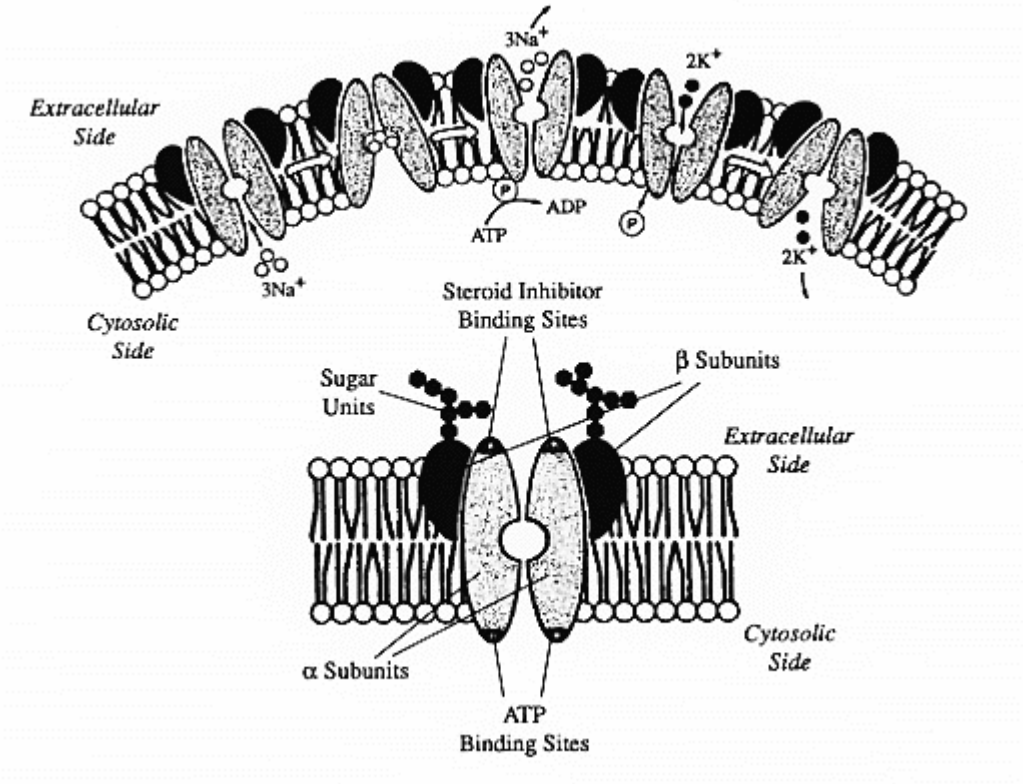
The practical biological and chemical implications of these isomeric structural differences is profound. D-glucose (dextrose) is the principal sugar used by the body to generate energy. By contrast, l-glucose cannot be significantly metabolized and an animal or human would starve to death if this was the only carbohydrate available in its diet and no other sources of energy (fats or proteins) were available. L-glucose looks, tastes and has the same mouth feel as d-glucose and there has been considerable interest in producing it in large quantities as an artificial sweetener. Unfortunately, the synthetic pathway to produce l-glucose, and more importantly, the separation of the d- and l-glucose isomers after synthesis is currently prohibitively expensive.

What does all this have to with cryonics and organ preservation? Under normal metabolic conditions the cells of the body produce chemical energy in the form of ATP and about 1/3rd of this energy is used to pump ions into and out of the cells. This is necessary because the most common salts (ions) are very small and can easily pass through the cell membranes. Two straightforward examples are very much on-point. Cells need high concentrations of the potassium ion inside them to be able to function properly including carrying out some vital chemical reactions and



doing things like contracting in the case of muscles or transmitting signals in the case of nerve cells. Conversely, cells must not have too much sodium in them or they become swollen (edematous) and while this can ultimately rupture or lyse the cell, long before this happens cell swelling disrupts the meshwork of supports that maintain the cell's shape and probably serve as scaffolding for various enzymes to be anchored on and to facilitate efficient chemical processing (metabolism). Unfortunately, sodium has a net negative charge and the protein inside cells has a net positive charge. Thus, sodium will flow into cells and carry water with it resulting in cellular edema. This process is prevented by active pumping of sodium out of the cell. Similarly, calcium is extremely toxic to cellular mitochondria in high concentrations and calcium is also used as a critical signalling molecule inside cells. Thus, the calcium concentration outside cells is typically 10,000 times higher than that present inside cells. Again, this difference in concentration is maintained largely by active pumping which requires energy expenditure and on-going metabolism.

So, sodium gets pumped out and potassium gets pumped in and this process is linked and carried out by the same molecular machine; the sodium-potassium pump.

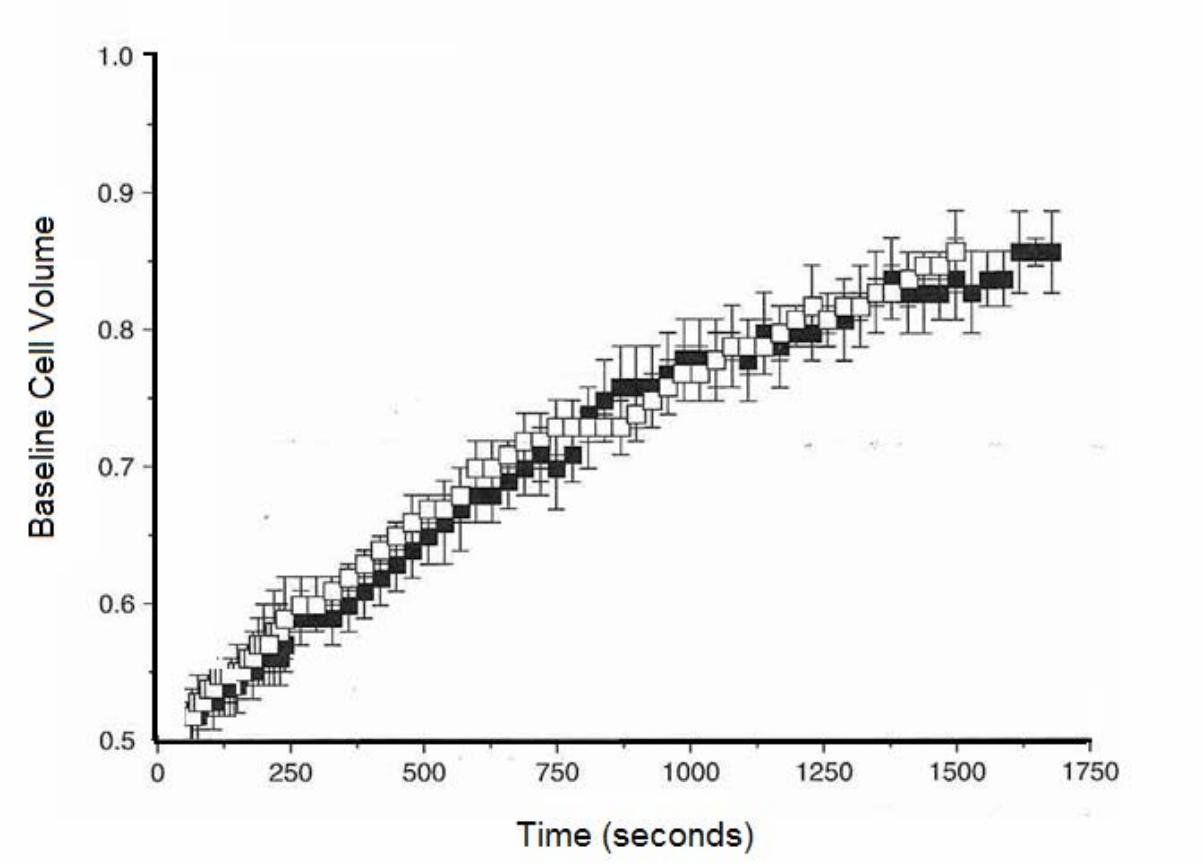


Of course, all of this presumes that there is both available energy in the form of ATP and that the cellular pumping machinery can use that energy. There are a number of things that can interrupt ion pumping. There can be a lack of energy due to starvation, hypoxia or ischemia, and there can exist situations where the energy is available but cannot be used. Some chemicals poison enzymes critical to ion pumping; a classic example is tetrodotoxin which comes from blowfish and which poisons sodium pumping. The other condition where adequate energy (ATP) can exist but cannot be used is deep hypothermia. Non-hibernating animals have enzymes that shut down or become inactive when the temperature is reduced well below that of normal body temperature. In humans (and most non-hibernating mammals) the enzyme responsible for pumping sodium out of cells and potassium into them, sodium-potassium-ATPase, is largely inhibited at 10^{o}C and is virtually shut down at few degrees above 0 ^{o}C

Cell swelling in brain cells occurs with incredible rapidity after interruption of blood flow in ischemia (cardiac arrest):

Brain (Glial) Cell Swelling in Global Cerebral Ischemia

(Cardiac arrest, rabbit)

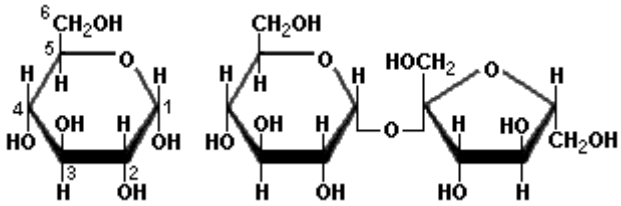


While cell swelling is not the only, or even primary, cause of injury in cerebral ischemia, it is a major player in causing injury in cold ischemia; conditions which obviously obtain in organ preservation and ultra-profound hypothermia in cryonics patients. The way this edema is prevented in organ preservation is to replace almost

all of the small cell membrane permeable ions with big molecules that cannot pass through the cell membrane and which are osmotically active; in others words can hold water outside of the cell. The first solution to do this with any success was Collin's Solution invented by Geoff Collins. It used comparatively large phosphate salts to keep water outside of the cells and prevent cellular edema. However, phosphates *do* leak across the cell membrane and they are incompatible with DMSO and also precipitate as crystals when solutions are cooled to low temperatures or frozen.

Thus, the organ preservationists turned to sugars and sugar alcohols as molecules to serve as an osmotic agent and prevent cell swelling. Sugars are comparatively large molecules and some are very large. They do not typically pass through cell membranes rapidly, if at all. Some of the first sugars tried were glucose and sucrose and the sugar-alcohol mannitol:

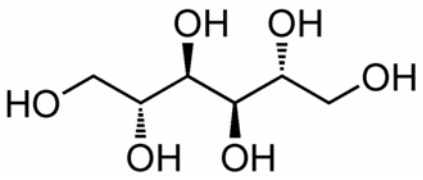
Glucose: Sucrose:



Neither glucose nor sucrose worked well. Glucose leaks across cell membranes fairly rapidly and has *facilitated transport* in the brain. Sucrose makes quite viscous solutions in the necessary concentrations (~180 mM) and for unknown reasons is toxic to the kidney tubule cells.

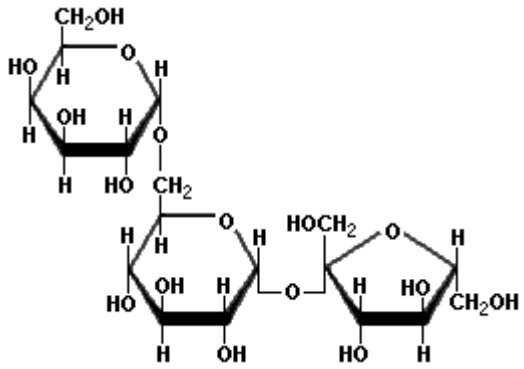
Mannitol was much more successful in the laboratory but never made it into clinical organ preservation solutions.

Mannitol:

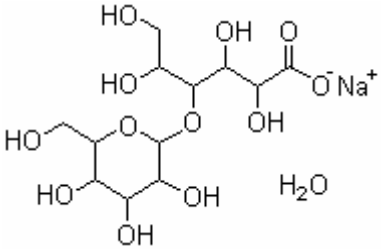


In the 1980s, a biochemist named Jim Southard and a transplant surgeon named Folkert Belzer began to systematically study molecules to inhibit cold ischemic swelling, as well as other molecules to help conserve ATP, inhibit free radical damage, and otherwise address the derangements that occur under deep hypothermic conditions. They identified two sugars as particularly effective in inhibiting cold ischemic cellular edema, raffinose and lactobionate.

Raffinose:



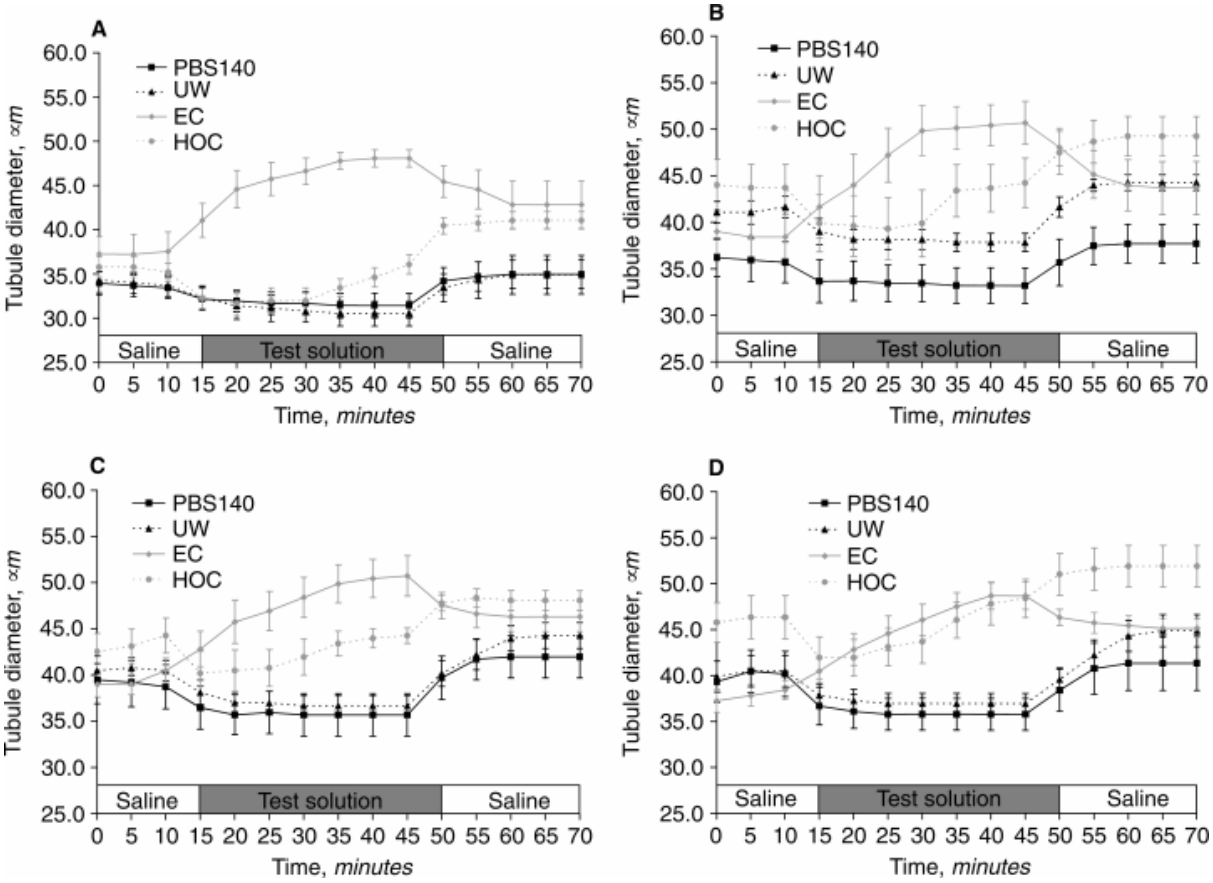
Lactobionate:



ViaSpan:

Component	*g/l*
Lactobionic Acid (as Lactone)	50.00
Potassium Phosphate monobasic	03.40
Magnesium Sulfate heptahydrate	01.23
Raffinose pentahydrate	17.83
Adenosine	01.34
Allopurinol	0.136
Total Glutathione	0.922
Dexamethasone	0.16
Glucose	0.90
Insulin (U-100 Regular)	40 IU
Heparin	2000 units/l
Water for Injection	q.s.
Potassium Hydroxide	q.s.
Sodium Hydroxide	adjust pH to 7.4
Measured Osmolality: 305 mOsm/kg	
Measured pH: 7.5	

Example of the Relative Effectiveness of Four Different Intracellular-Type Organ Preseervation Solutions at Inhibiting Cellular Edema



By using the strophanthidin-based model, a comparison (*A*) of the commercially available solutions with a phosphate buffered sucrose solution (PBS140) showed that cell swelling was minimized by either University of Wisconsin (UW) or PBS140 compared to Euro-Collins (EC) and hyperosmolar citrate (HOC) at time 0($P < 0.01$). The effect of 24 hours of cold ischemia (*B*) showed that cell swelling was most effectively prevented during a warm ischemic episode if PBS140 was used ($P < 0.01$). After 48 hours of cold ischemia (*C*), our data show prolonged protection with PBS140 comparable with UW. Prolonged protection with PBS140 comparable with UW was also seen at 72 hours of cold ischemia (*D*).

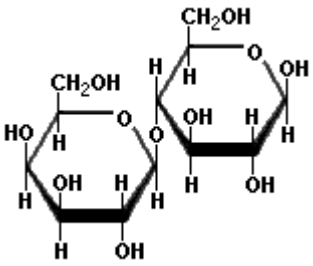
They combined these two sugars along with other ingredients to create the first and still most successful “universal” organ preservation solution, UW-Solution, or as it is commercially marketed, Viaspan.

Unfortunately, ViaSpan does not work for the brain. We tried it extensively in the early 1990s and got serious cerebral edema followed by convulsions and death in dogs that had been perfused with ViaSpan for as little as two hours! By contrast, we could recover dogs perfused with MHP (a mannitol based perfusate) after 5-hours of bloodless perfusion with the solution at 5^{o}C with no neurological or other problems;

most of the dogs were placed with cryonics members and lived out the rest of their lives normally.

Recently, 21st Century Medicine has been systematically investigating hypothermic organ preservation and they have made a number of stunning breakthroughs. One thing which was long overdue to be done was to systematically screen various molecules for their cell swelling inhibiting effects. They found that one sugar in particular was highly effective, D(+)-lactose. Only the d-isomer worked well.

D(+)-Lactose:



Why some sugars work and others do not, or actually cause harm, is a mystery. The molecular weight is certainly a factor, but different sugars with nearly identical molecular weights may perform totally differently. Also, the isomer of the sugar appears critical in some cases, as is the situation with lactose. 21st Century Medicine has patented an organ preservation based on D(+)-lactose and is in Phase II clinical trials for this solution, which they call TranSend. They are currently getting 72-hour simple flush and store on ice preservation of kidneys (rabbit and dog), pancreases and livers (dogs) and are getting similar results in the human clinical trials. They have achieved 48-hour heart preservation using a derivative of this solution which combines periods of trickle-flow cold perfusion with brief intervals of modest warming to ~15 ^{o}C.