16. Remote Blood Washout

This section consists of two parts. In Part 1, the rationale and practice of remote blood substitution is reviewed. In Part 2, the history and conduct of extracorporeal perfusion is reviewed.

Part 1

In an "ideal" cryonics case the patient is pronounced legally dead as close to the cryonics facility as possible to minimize the period between cardiac arrest and long term care after vitrification. In contemporary cryonics, local stabilization is only possible in a minority of cases, and in remote cases there can be situations in which no stabilization is possible (i.e., sudden death, autopsy, no standby).

When remote standby and stabilization procedures are possible, the current protocol is to wash out the blood of the patient and substitute it with an organ preservation solution prior to air shipment or ground transport. Although the desirability of remote blood substitution goes back to the first cryonics transport manual in 1972, the routine practice of blood substitution in cryonics goes back to the early 1980s, and reflects findings in hypothermic resuscitation research and clinical hypothermic organ preservation. Since then a substantial number of cryonics patients have undergone remote blood washout prior to shipment to a cryonics facility.

Over the last ten years there has been increasing concern that blood washout is counterproductive, or should be restricted to only some cases, or should be undertaken only if better preservative solutions are developed and validated in a model clinically relevant to cryonics patients. Some cryonics observers have expressed concern about performing blood substitution in patients after long periods of (cold) ischemia and/or long periods of

cardiopulmonary support because of the prospect of serious reperfusion injury and related concerns such as edema or aggravating injury to the patient's circulatory system.

The current remote blood washout procedures are supported by the canine asanguineous ultraprofound hypothermia research that Michael Darwin, Jerry Leaf et al did in the mid-1980s[1]. In these experiments dogs were resuscitated from up to five hours of low-flow asanguineous ultraprofound hypothermic circulation (temperature <10 degrees Celsius). This model raises a number of questions. The most obvious question is whether these results warrant extrapolation to longer periods of low-flow perfusion and circulatory arrest. In typical cryonics patients, the period between blood substitution and long term care exceeds the period of asanguineous hypothermia in the experiments mentioned above. And with the exception of a small number of older cryonics cases, current patients are *not perfused during transport* to the cryonics facility. Barring actual experiments, it cannot be assumed that blood substitution of cryonics patients, followed by periods of 24 hours or more of circulatory arrest during transport, is superior to no blood substitution or remote extracorporeal bypass without blood substitution. This concern is further justified by the fact that the original canine experiments were done on *non-ischemic healthy* animals, whereas cryonics patients are almost invariably aged, fragile and ischemic, and even under the best conditions experience severe agonal injury.

Another concern that has been raised involves the composition of the perfusate. There is now documented evidence that recent perfusate preparation and composition differs from the clinical considerations that informed perfusate composition during the original canine experiments and subsequent application of these solutions to cryonics patients and earlier cryonics cases. A related issue is that human cryopreservation might benefit from validation and introduction of a new generation of cold organ preservation solutions and/or new protocols. The MHP-based cold organ preservation solutions are now more than 30 years old, and research in and outside of cryonics has developed new cold organ preservation solutions that may be superior to MHP-based perfusates. For example, one design consideration for cold organ preservation

solutions for *cryonics patients* would be to include neuroprotective agents in the perfusate.

A final concern that will be addressed is whether the current state of expertise at Alcor (or associated organizations) warrants routine remote blood substitution. Remote blood substitution is a non-trivial procedure, requiring surgical skills and operation of a portable heart-lung machine, and can cause great harm to a cryonics patient if not executed correctly.For this reason, since 2011 Alcor has had a policy using the contractor Suspended Animation, Inc. (SA) to perform blood substitution of Alcor patients in the continental United States outside of Arizona. SA in turn contracts with cardiothoracic surgeons and clinical perfusionists, who are medical experts in accessing large blood vessels and diluting or replacing blood.

Why Blood Substitution?

There are three important reasons for remote blood substitution:

1. Elimination and prevention of blood clots.
2. Rapid induction of ultraprofound hypothermia.
3. Maintaining viability of the brain during transport to the cryonics facility

Two related objectives are the elimination of inflammatory products in ischemic patients, and maintaining circulation after cardiopulmonary support.

Circulatory Arrest and Blood Clotting

Perhaps the most fundamental reason to replace the blood with an organ preservation solution is to eliminate blood clots and to prevent blood clotting (or related blood abnormalities). The reason for this is obvious; if a patient suffers from massive micro and macro- blood clotting, subsequent attempts to perfuse the patient with a vitrification agent will be sub-optimal or fail completely.

Despite this obvious objective, is not clear why circulatory arrest should necessarily induce blood clots. Scientific discussion of the relationship

between circulatory arrest and blood clotting is rare. This lack of scientific literature on this issue is also problematic in light of the fact that clotting can be unpredictable; clots sometimes are observed in cryonics patients, but other times perfusion is apparently successful after long ischemic times even without heparin administration. These anecdotal observations should be qualified by stressing that the lack of visible clotting and the ability to cryoprotectant such patients, does not rule out the absence of harmful micro-thrombi which can leave parts of the brain non-perfused. Areas of the brain that are shut off from circulation risk straight freezing, defeating the purpose of vitrification and increasing the demands on future resuscitation technologies.

Most models that have investigated coagulopathy in shock, cardiac arrest, and stroke *restore circulation* after various periods of ischemia (or low perfusion) and measure coagulation times and various plasma concentrations of blood components such as fibrogen and platelets. Hossmann et al. induced 1 hour of normothermic cerebral ischemia in cats and found reduced electrophysiological recovery from prolonged normothermic recovery in animals with lower post-ischemic fibrinogen concentrations[2]. Similarly, Cerchiari et al. observed hypocoagulability after 7.5 to 12.5 minutes of cardiac arrest in dogs, indicating hypercoagulability and disseminated intravascular coagulation (DIC) early after cardiac arrest. Böttinger et al. investigated hemostatic changes in humans during cardiopulmonary resuscitation (CPR) and found marked activation of blood coagulation and fibrin after prolonged cardiac arrest and CPR without concomitant activation of fibrinolysis[3]. The authors propose that these events contribute to the post-resuscitation “no reflow” phenomenon, in which some areas of the brain do not perfuse.

In contrast, Fisher et al. designed an elegant experiment in which rabbits were perfused with carbon black after various durations (4.5, 15, and 30 min) of cerebral ischemia and different reperfusion protocols[4]. They found no difference between untreated animals and animals pre-treated with heparin after 15 minutes of ischemia. In both groups cerebral perfusion was impaired. In animals that were re-perfused with increased pressure or hemodiluted with saline, less perfusion impairment was observed. This study indicates that formation of thrombi does not occur within the durations of ischemia that

were studied. But perhaps the conclusions from Böttinger et al. and Fisher et al. can be reconciled if we allow for the possibility that cerebral ischemia *does* induce formation of *micro-thrombi* but these are not of such a magnitude that cerebral circulation is greatly impaired. Tisherman et al. have observed large vessel blood clots in rats and dogs after 20 minutes of normothermic cardiac arrest induced by drowning[5]. The success of hypertension and hemodilution in mitigating the no-reflow phenomenon does indicate that rheological and vascular problems are involved in impaired perfusion after cerebral ischemia. For example, Safar et al. obtained better cerebral outcome after 12 minutes of cardiac arrest in dogs using hypertensive reperfusion, heparin, and dextran administration[6]. In recent research conducted at Advanced Neural Biosciences in the rat model administration of heparin (and streptokinase) was not sufficient to prevent perfusion impairment and prolonged periods of warm and cold ischemia.

Although it is often taken for granted that blood clots form after cardiac arrest, the mechanisms for this are rarely discussed. Since blood stasis by itself does not activate the *intrinsic* or *extrinsic* clotting cascade, some elucidation of these mechanisms in the context of cryonics would be helpful. One proposed explanation is that red blood cells modify their cytoskeleton when they stop moving. The cytoskeleton becomes more rigid when the laminar flow through the vessels is lost, exposing phospholipids at the membrane surface, which trigger the coagulation cascade[7]. This raises the question of whether blood clots during stasis or whether thrombi are formed during reperfusion because of failure of the fibrinolytic system. In contrast, in forensic medicine it seems to be common knowledge that blood becomes “permanently incoagulable” within 30-60 minutes of death[8]. In a recent book length treatment of deep venous thrombosis (DVT), P. Colm Malone and Paul S. Agutter conclude that blood cannot coagulate in a cadaver and that all thrombi (which the authors carefully distinguish from in vitro clots) are agonal in nature. The “mode of death” framework they present allows the authors to explain why thrombi are found in some cadavers but not in others. In the case of *sudden* circulatory arrest we would not expect much benefit from “post-mortem” anti-thrombotic therapy, whereas in the case of gradual and selective circulatory failure (shock) we would expect increased thrombi formation[9].

As the work of Fisher et al. indicates, leaving the blood in the patient may present other challenges than blood clotting such as platelet activation, adhesion and rolling of white blood cells, cold-induced red cell aggregation, and hyperviscosity. Studying the mechanisms of all these phenomena in the context of cryonics will be a formidable task. The most realistic direction so far has been to design experiments to investigate the difference between various remote stabilization protocols with quality of (cryoprotectant) perfusion as an endpoint. The most fundamental question in this context is to investigate if there is a difference between the quality of perfusion in models with and without blood washout, looking at various lengths of cold ischemic storage times, and comparing different organ preservation solutions.

Rapid Induction of Ultraprofound Hypothermia

One clear advantage of remote blood substitution is that obtaining access to the circulatory system enables the cryonics stabilization team to induce internal cooling, which produces superior cooling rates compared to any other method of (external) cooling. Strictly speaking, extracorporeal cooling does not imply blood substitution. If it would be established that the other advantages of remote blood substitution are absent, or even counter-productive, the portable heart-lung machine could just still be used to induce ultraprofound hypothermia (core temperature between 0 and 5 degrees Celsius) without washing out the blood and substituting it for an organ preservation solution. In the future, cold cyclic lung lavage could take over this remaining use of extracorporeal perfusion, provided it can achieve comparable cooling rates and that prolonged CPS at low temperatures is not detrimental. Since the advantages of rapid induction of hypothermia in cryonics are not controversial, this (indirect) advantage of remote blood substitution will be assumed as a given in this chapter.

Maintaining Viability

Another important objective of blood substitution is to maintain viability, and that of the brain in particular, during cold transport of the patient. The assumption is that an artificial “whole body” preservation solution will limit ischemic injury to a greater extent than leaving the blood in the patient.

Ischemia-induced edema can occur during stabilization, total body washout, and transport, and becomes acute upon initiation of CPA perfusion. This edema can be so severe both systemically and in the brain that it precludes completion of CPA perfusion. Another serious complication is fulminating pulmonary edema, stomach and gut leakage of perfusate that either exhausts the available stock of perfusate or, in some cases, is so severe it precludes the maintenance of adequate arterial perfusion pressure. This latter complication presumably reflects severe injury to the integrity of the capillary endothelium and to the underlying basement membrane.

The question in cryonics is whether it is reasonable to extrapolate evidence of superior ultrastructure and viability achieved with asanguineous ultraprofound hypothermic arrest in healthy animals to cryonics patients who are (often) old, ischemic and exposed to much longer times of hypothermic circulatory arrest. Before reviewing this issue it will be good to briefly review the state of the art in mainstream hypothermic organ preservation and asanguineous hypothermic resuscitation.

Hypothermic Organ Preservation

The history of hypothermic organ preservation is closely linked to the history of organ transplantation. To maintain viability between the time of organ harvesting and transplantation a number of techniques can be deployed ranging from warm perfusion to asanguineous cold storage.

The most basic perfusate is no perfusate at all, but rather hypothermic perfusion of isolated organs with whole blood. Although organs have been preserved in this manner, there are some major disadvantages to this method. Cold induces red cell sludging and during hypothermia hemoglobin holds oxygen so tightly that it gives up little oxygen. Other alternatives include plasma, cryoprecipitated plasma and perfusates of serum or fractions of serum. Because these alternatives still suffer from issues such as the presence of unstable lipoproteins, clotting, precipitation, cytotoxic antibodies, and contamination issues, improved perfusates were designed that were completely synthetic and a-cellular in nature. Perfusates can either contain a colloid (like albumin) or no colloid. The most popular cold organ preservation

solutions today contain artificial colloids and a number of components to provide metabolic support, stabilize pH, prevent edema, protect against free radical injury, and protect against cold cell swelling.

The organ preservation solution that Alcor uses today, MHP-2, is an "intracellular" type cold organ preservation solution. MHP-type solutions are similar to Viaspan, also known as University of Wisconsin solution (UW solution), still the "gold standard" for organ preservation solutions. Intracellular cold organ preservation solutions mimic the intracellular ionic environment. Although lower temperatures will reduce the rate of biochemical reactions, the rates of *biophysical* events (such as diffusion) do not decrease at the same rate. Cold impairs ATP-driven ion pumps in cell membranes, but passive transport continues as ions move down their electrochemical gradients causing membrane depolarization, intracellular calcium overload, cell swelling, and ultimately, cell death. To prevent such events, intracellular cold organ preservation solutions are high in potassium (hyperkalemic) and low in sodium. This ensures that the intracellular environment remains in its natural high potassium state as diffusion equalizes intracellular and extracellular ion concentrations during failure of ion pumps in cell membranes.Cold preservation solutions also derive most of their tonicity from relatively large impermeable solutes such as mannitol.This is necessary to prevent "colloid osmotic cell swelling."Free diffusion of small ions into cells during failure of cell membrane ion pumps leaves large impermeable intracellular anions (negatively charged proteins or "colloids") as the most effective osmotic agents when cells are cold.If liquid surrounding cells contains only small permeable ions such as sodium, potassium and chloride when ion pumps fail, then the impermeable anions inside cells osmotically draw water into cells and cause cells to swell.The inclusion of large impermeable osmotic agents such as mannitol or lactobionate in organ preservation solutions counterbalances the osmotic effect of large anions inside cells, and prevents cell swelling that would otherwise occur while membrane ion pumps are impaired. Other components that are important to MHP-type and UW solutions are hydroxyethyl starch, which by being impermeable across capillary walls provides colloid osmotic support to keep blood vessels open and prevent edema, and glutathione to scavenge free radicals. As will be discussed later,

the major difference between MHP-2 and Viaspan is that the former includes the impermeable component mannitol and the latter the more expensive sugars lactobionate and raffinose. MHP based cold organ preservation solutions were designed to reflect the state of the art in cold organ preservation at the time (1980s) and to allow for hypothermic asanguineous resuscitation.

Hypothermic Perfusion Preservation

Although hypothermic perfusion preservation (HPP) was an important modality of organ preservation, this technology has been increasingly replaced by static hypothermic storage because of logistics, cost, and improvement in cold storage solutions such as UW solution. One major limitation of hypothermic static storage is that there are finite limits to the length of time organs can be maintained at low temperatures without running into energy shortages and associated cell death. HPP has an advantage over static storage because it can deliver cell nutrients to the tissues and remove CO2 and other metabolic waste products. Hypothermic perfusion also allows for reversal of the early stages of warm ischemia such as edema and microcirculatory failure. The physiological benefits of HPP include sustenance of mitochondrial electron transport, reduction of apoptosis, improved circulation after warm ischemia, and the ability to actively regulate pH and administer cytoprotective and immuno-modulating drugs[10].

Most potential negative features of HPP mirror the positive features of the technology. Although HPP can improve preservation of ischemic organs, it can also worsen them by exerting high pressures, high viscosity, and excessive shear stress. This risk is particularly important in cryonics because most cryonics patients experience long agonal periods, shock, cardiac arrest, and hypoperfusion during cardiopulmonary support. Another potential disadvantage of HPP is the risk for increased interstitial and cellular edema when organs are perfused with solutions without adequate oncotic support. This presents another major risk for cryonics patients because such events can frustrate later attempts to cryoprotectant the brain. The practice of continuous (vs. intermittent) perfusion in cryonics, and the choice of the right oncotic agents will be discussed in more detail below.

Hypothermic Preservation of the Isolated Brain

Research into hypothermic preservation of the *isolated brain* is very rare. The most important reason for this is that unlike other organs, the brain is the seat of the identity of the person and cannot be swapped for another brain without changing persons. Although it may become technically feasible to replace the body of a critically ill patient with another body, such a procedure raises technical and bioethical challenges. One researcher who explored "brain transplants" and brain preservation is Dr. Robert J. White (1925-2010). Obviously, studies of hypothermic preservation of the brain are of great importance to cryonics and the practice of remote blood substitution in particular.

In 1963, White et al. *perfused* an isolated monkey brain in vitro at hypothermic temperatures and observed persistence of electrical activity for periods ranging from 30 to 180 minutes[11]. The paper does not mention oxygenation or the use of asanguineous perfusion, only that perfusion pressure was maintained by "the addition of small increments of dextran or compatible donor blood." Brain temperatures measured were between 30 degrees Celsius and 35 degrees Celsius. In 1964, White el at. reported persistence of electrical activity in the isolated brain being perfused with a complete mechanical extracorporeal system incorporating a small disk oxygenator[12]. The researchers observed increasing lactate and pyruvate (indicating developing acidosis) after 2 hours and cerebral edema after 3 hours. Electrocortical activity gradually declined after 3.5 hours. In 1966, White et al. investigated refrigeration of the whole canine brain in vitro at ~2-3 degrees Celsius[13]. The blood in the brains was replaced by heparinized Ringer's lactate solution. Brains that were stored up to 4 hours showed electrical activity after temperatures of 22 degrees Celsius or more were reached during reperfusion. Prolonged maintenance perfusion of the rewarmed brain exceeding 6 hours after hypothermic storage, however, resulted in the disappearance of electrical activity and irreversible edema. Most remarkably, White et al. report "excellent revascularization of the brain" during perfusion, even in the 2 brains that had been stored for 15 (!) days. They do report increased edema for brains with longer storage times, but this edema could be delayed and

temporarily offset by increasing the osmolality of the blood by adding urea. The latter observation has important consequences for the design of organ oreservation solutions and cryoprotective carrier solutions in human cryopreservation. In 1972, White et al. reported persistence of electrical activity in isolated brains that underwent low flow asanguineous perfusion for 4 to 6 hours below 5 degrees Celsius[14].

These results indicate that the viability of the whole brain (as evidenced by recovery of electrical activity after rewarming) can be maintained for up to 4 hours after static cold storage and up to 6 hours after low flow asanguineous perfusion. Two important caveats are in order, however. Firstly, as reported by White et al. long-term persistence of electrical activity after long term ultraprofound storage appears to be a challenge. Secondly, isolated brain studies constitute somewhat of an “ideal” research model. And an additional caveat specific to cryonics is that the brain of a typical cryopatient is moderately to severely ischemic prior to hypothermic preservation. Having said this, the results by White et al. are consistent with the results (and limits) obtained in whole body ultraprofound asanguineous circulatory arrest and perfusion.

Normothermic Isolated Brain Perfusion

Like conventional organ preservation, perfusion of the isolated brain is often done at hypothermic temperatures. The most important advantage is that the adverse effects of isolated perfusion will be mitigated by low temperatures. If the isolated brain is perfused for a prolonged period of time, damage to blood cells will lead to increasing hypoxia and impairment of circulation, with reduced viability as a result. Despite these limitations, the need for normothermic isolated brain perfusion models has been recognized. Normothermic isolated brain perfusion can be helpful when the specific effects of administration of a substance of the brain need to be studied without the interference of the rest of the body, and the action of other organs on the body, in particular. The model can also be used to investigate cerebral ischemia, the blood brain barrier (BBB), and the effects of different flow rates and energy substrates on the brain. Perfusing the brain at normal body temperature will eliminate the effects of cold on biochemical reactions and

specific effects of hypothermia. Currently, the challenges of warm isolated brain perfusion are addressed by using oxygen-carrying compounds such as perfluorocarbons in combination with synthetic buffers such as HEPES as the perfusate.

Although such models are of limited use in predicting what to expect in hypothermic organ preservation and whole body asanguineous ultraprofound hypothermia, let alone remote blood substitution of cryonics patients, they can be used to study specific phenomena and to screen agents for use in cryonics. Mukherji et al. review the use of this model to look at brain metabolism after administration of various compounds[15]. Some findings they present include the observation that mannose can completely substitute for glucose as the energy substrate, ethanol is not metabolized in the brain, and dimethyl sulphoxide (DMSO) increases the rate of glycolysis. Recent studies with isolated brain preparations have been used to investigate the preservation of the blood brain barrier and the molecular control of micro vessels. Such models could be particularly helpful in studying the (specific) toxicity of cryoprotectant agents and its effects on the vasculature of the brain.

Isolated Head Perfusion in Cryonics

It should be noted that a related procedure to isolated perfusion of the brain that exists as a clinical procedure in cryonics is *isolated head perfusion*. To reduce (surgical) time and avoid injury to the brain, in cryonics the brain is left in its skull while it is being perfused with a vitrification agent. Isolated head perfusion offers several advantages to including the rest of the body (or upper body) during perfusion of the brain. The most obvious advantage is that cephalic isolation prior to cryoprotective perfusion reduces the time between start of surgery and start of cryoprotectant perfusion. This is especially beneficial in cases where the patient presents at relatively high body temperatures. A related advantage is that cannulating only the head does not require the additional step of clamping off the descending aorta and the extremities. Because most of the cross-section of the stump is available for venous drainage, isolated head perfusion should also present fewer pressure related complications during perfusion. Typically, central venous pressure tends to rise during the final stages of cryoprotectant perfusion, shunting a

portion of the perfusate through the (normally) higher resistance bridging veins. As a consequence, less burr-hole drainage and facial edema has been observed during isolated head perfusion.

Whole Body Hypothermic Resuscitation

The gold standard for evaluating the efficacy of asanguineous ultraprofound hypothermia is the ability to resuscitate the person (or animal in a research) from a state of low-flow perfusion or specific periods of complete circulatory arrest. Being able to demonstrate this capability would provide evidence that the initial part of cryonics procedures—cooldown to low temperatures—just above zero degrees Celsius, is reversible with contemporary technologies. Because most cryonics patients will generally not be transported to a cryonics facility while undergoing hypothermic bypass, the most relevant research model is one of profound or ultra-profound whole body circulatory arrest. Induced deep hypothermic circulatory arrest is now routinely used in conventional medicine for procedures where the surgeon needs a motionless and bloodless field. Profound and ultra-profound (asanguineous) circulatory arrest are currently being investigated as a means to treat trauma victims and to protect the brain from injury after normothermic cardiac arrest.

Like in vitro hypothermic organ preservation, the length of time experimental animals can be held at low temperatures (sometimes close to the freezing point of water) depends on whether the blood is substituted with a suitable “global” whole body perfusate and whether the animal is in a state of complete circulatory arrest or receiving low flow perfusion. The current documented record for recovery from circulatory arrest without significant brain damage stands at 3.0 hours near 0°C in a canine model[16] . The current documented (but unpublished) record for recovery from low flow ultraprofound asanguineous hypothermia is 5 hours in a canine model[17].

These records seem to be consistent with the best results White et al. achieved for cold storage (up to 4 hours) and perfusion of the isolated brain (up to 6 hours), especially if one allows for the fact that the isolated brain model is the “ideal” research model and complete recovery of the experimental animals without adverse neurological effects constitutes a

stricter definition of successful resuscitation than demonstration of maintenance of electrical activity in the brain after 3.5 hours of circulatory arrest. Harris notes that if we estimate the equivalent normothermic ischemic time for a dog at 3.0 hours of circulatory arrest at temperatures just above the freezing point of water we find a value of ~10 minutes at normal body temperature[18]. Complete neurological recovery from 10 minutes of normothermic circulatory arrest can be reconciled with the current 5 minute limit for dogs and humans if we take into account a number of important differences between conventional normothermic resuscitation and asanguineous ultraprofound resuscitation.

Perhaps the most important difference is that the blood of the experimental animals was replaced with an “intracellular” solution that is designed to counter the adverse effects of hypothermia and ischemia. Such organ preservation solutions often contain agents that have neuroprotective properties such as glutathione and mannitol. Therefore, these models should be compared with models of normothermic resuscitation in which neuroprotective agents are administered. Darwin, Harris et al. were able to resuscitate dogs from more than 16 minutes of normothermic cardiac arrest employing an aggressive post-resuscitation drug protocol and management of hemodynamics[18]. Extrapolating these normothermic results to an equivalent time of circulatory arrest at temperatures close to zero degrees Celsius indicates that resuscitation from ultraprofound asanguineous circulatory arrest might be possible for up to 5 hours, provided the perfusate includes similar cerebroprotective agents as were used in the normothermic experiments of Darwin and Harris. Furthermore, the animals in the ultraprofound asanguineous circulatory arrest experiments may also benefit from the fact that they were kept cool for hours after the experiment. Such a protocol would mimic a model of normothermic circulatory arrest plus post-resuscitation hypothermia. In light of the observation that even very modest decreases of brain temperature can have a profound neuroprotective effect, the value of 2.2 for Q10 may be too conservative. For example, Nakashima et al. observed different temperature sensitivities for release of the neurotransmitters glutamate, aspartate, glycine, and GABA during cerebral ischemia[20]. Such findings may indicate that even longer periods of circulatory arrest at

hypothermic temperatures might be feasible. On the other hand, the rate of biophysical events (such as diffusion of water and ions) is not as affected by temperature as biochemical events, limiting the efficacy of hypothermia as the sole treatment prior to inducing circulatory arrest.

A number of observations about past and recent whole body hypothermic resuscitation studies in the context of cryonics are warranted. That the superiority of asanguineous perfusion or circulatory arrest over hemodiluted, let alone whole blood, is taken for granted in most of these studies is evidenced by the fact that it is hard to find studies that compare the outcome of non-asanguineous whole body hypothermic resuscitation to asanguineous resuscitation in terms of neurological behavior and histology. Although the assumption seems to be that the superiority of bloodless isolated organ preservation does not warrant such experiments, this state of affairs makes it difficult to compare asanguineous to non-asanguineous experiments. For example, the Alcor asanguineous ultraprofound hypothermia experiments solely consisted of a number of experiments using identical protocols and perfusate composition. In light of the fact that these experiments were conducted to improve technologies for remote stabilization of cryonics patients, the absence of any non-asanguineous controls results in reduced context, and a missed opportunity to elucidate the mechanisms of injury when the blood is left in the animal. More recent experiments by other groups also lack such controls. It is also worth mentioning that the current documented record of 3 hours for whole body hypothermic resuscitation did not involve complete blood substitution of the animals[21]. In addition, the successful hypothermic whole body resuscitation experiments in rats and hamsters that were conducted during the middle of the 20th century by researchers such as Smith and Andjus did not include blood substitution but were still effective in restoring full body recovery after more than 3 hours of ultra-profound cold ischemia. In an important paper, Sekaran et al. addressed the necessity of blood substitution in detail by comparing neurological recovery in a porcine model of ultraprofound hypothermic circulatory arrest[22]. The researchers divided the animals into one of three target hematocrits groups (0%, 5%, and 15%) during circulatory arrest and observed significantly better neurological recovery in the group that underwent complete blood replacement (hematocrit

0%). The authors also discuss a study in piglets[23] where the opposite was observed, but point out that the higher temperature in this study (15 degrees Celsius) may have avoided the deleterious effects on blood components that is observed when the temperature is dropped lower than 5 degrees Celsius. The Sekaran study strengthens the case for remote blood substitution in cryonics although two caveats are warranted. First, superior results obtained in bloodless ultraprofound hypothermia cannot be just extrapolated to cryonics patients, who typically have experienced significant ischemic injury prior and/or after legal death. Second, it remains to be investigated if the superior benefits of asanguineous ultraprofound hypothermia can be "mimicked" by pharmacological treatments without conducting blood substitution.

Composition of Whole Body Organ Preservation Solutions in Cryonics

Before discussing the indications and contra-indications for remote blood substitution in cryonics, it will be helpful to briefly review the history and the composition of the organ preservation solution that is currently used in cryonics. This will not only set the stage for discussion of improved organ preservation solutions, it will also help with reconciling the experimental results obtained in whole body washout canine experiments and observations made during cryonics cases.

The current organ preservation solution used by cryonics organizations is called MHP-2. MHP-2 stands for Mannitol Hepes Perfusate number 2. MHP is a so-called "intracellular" organ preservation solution because its ionic composition mimics that of the cell to counter hypothermia-induced cell swelling. MHP itself was inspired by Gregory Fahy's RPS-2, Renal Preservation Solution number 2. RPS (itself a modification of Euro-Collins solution) is a hyperkalemic intracellular organ preservation solution designed for hypothermic preservation of kidneys. The published formula for RPS-2 is: 7.2 mM K2HPO4; 5 mM reduced glutathione; 1mM adenine HCl; 180 mM dextrose; 28.2 mM KCl; 10 mM NaHCO3; 2 mM Ca2+ and 1 mM Mg2+.

RPS-2 was modified to be used for asanguineous ultraprofound resuscitation. The most important changes include a substantial reduction of

glucose in favor of the impermeant osmotic agent mannitol because high concentrations of glucose produced severe acidosis during whole body hypothermic blood substitution. The other major change is the substitution of HEPES (N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid) for the phosphate based buffer. HEPES is compatible with higher pH's of the perfusate (which is deemed to be beneficial at lower hypothermic temperatures) and does not cause precipitation of calcium and magnesium salts. MHP also includes a colloid to counter development of edema during prolonged hypothermic perfusion. Michael Darwin and Jerry Leaf observed that other colloids produced edema in the lungs and pancreas (Dextran 40) and acute lesions in the liver (polyvinylpyrrolidone: PVP). MHP was meant to be introduced with 1000 I.U. (International Units) of Heparin to prevent coagulation.

Although the qualitative differences between RPS-2 and the MHP based solutions are known, it has turned out to be a considerable challenge to reconstruct the "official" formulas for MHP and MHP-2. One reason for this is that various formulations have been published in Alcor's Cryonics magazine, the official write-up of the hypothermic canine experiments, and the Leaf & Darwin patent[24]. The preferred formulation in the patent and the July 1984 issue of Cryonics magazine agree on all the components, except for adenine and glutathione, which are omitted in the preferred formulation in the patent and the table that documents the composition of the total blood washout solution used in the canine experiments[25]. It can be assumed that the amounts for adenine and glutathione documented for MHP in the July 1984 issue of Cryonics magazine are identical to those in RPS-2. Whether these two components are part of the official formula of MHP depends on whether one takes the published formula in the magazine or the formula published in the canine experiments as the standard. Because the author has not been able to find an official announcement about MHP-2, its formulation can only be deduced by comparing the components and values for MHP with those for MHP-2 (as used in cryonics cases and experiments). The most fundamental change was the addition of D-ribose. No official announcement has been made to add D-ribose to Alcor's washout perfusate but the desire to modify the MHP perfusate to prevent development of skeletal and cardiac muscle rigor is

evident in the discussion of the 16th total blood washout canine experiment, in which a modified UW solution was used as a flush solution[26]. In a case report for the May 1989 issue of Cryonics magazine Mike Darwin states that "we also think it possible that the addition of phosphate and ribose to the flush perfusate resulted in better metabolic support to the muscles (and presumably neurons and other body cells) during the subsequent cold ischemia of air transport." Presumably, for these reasons D-ribose is the additional component in MHP-2. Other changes include reductions in the amounts of adenine and glutathione, a (further) decrease of the amount of glucose by 50%, and doubling of the concentration of HEPES. Unlike the original MHP patent and the total body blood washout article, published formulas of MHP-2 also include insulin. The rationale for insulin (and other remaining uncertainties) in cryonics organ preservation solutions will be discussed in more detail below. No documented attempts to improve upon MHP-2 exist since this statement of almost 25 years ago.

A comparison between the RPS-2, MHP, and MHP-2 can be found in the table below:

	RPS-2	**MHP**	**MHP-2**
Mannitol	-	170 mM	170 mM
Adenine-HCL	1 mM	(1 mM)	0.94 mM
D-ribose	-	-	0.94 mM
Sodium bicarbonate	10 mM	10 mM	10 mM
Potassium phosphate	7.2 mM	-	-
Potassium chloride	28.2 mM	28.3 mM	28.3 mM
Calcium chloride	1 mM	1 mM	1 mM
Magnesium chloride	2 mM	1 mM	1 mM
HEPES	-	7.2 mM	15 mM

Glutathione	5 mM	(5 mM)	3 mM
D-Glucose (Dextrose)	180 mM	10 mM	5 mM
Hydroxyethyl starch	-	50 g per L	50 g per L
Heparin	-	1000 I.U. per L	1000 I.U. per L
Insulin	-	-	40 I.U. per L
Osmolality	280-290 mOsm	388-403 mOsm	388-403 mOsm
pH	7.4	8.0-8.2	8.0-8.2

Logistical and Technical Aspects of Total Body Washout

The most important reasons to question the practice of remote blood substitution (prior to transport) in cryonics are concerns about the effect of blood washout in ischemic patients and the alternative of conducting field cryoprotection instead. In a number of cases, (cerebral) edema has been observed in patients whose blood was replaced with an organ preservation prior to cold transport. As will be discussed below, at the Cryonics Institute, research by Yuri Pichugin did not find better viability in brain slices that were preserved in an organ preservation vs. brain slices that were preserved in whole blood. These results seemed to be consistent with the results by White et al. that it is not likely that brains can be kept viable for periods exceeding 4 hours of cold storage. Additional concerns about the lack of proper equipment at funeral homes and reperfusion injury contributed to the decision at the Cryonics Institute not to do remote blood substitution for cases in which the patient does not receive rapid stabilization. However Advanced Neural Biosciences, Inc. (ANB) has done research that found improved cryoprotectant perfusion of rat brains after up to 48 hours of cold storage following blood washout with MHP-2 compared to rats to which only anticoagulants were administered. Before we address the topic of reperfusion

injury a number of other reasons for forgoing remote blood substitution are briefly mentioned.

Logistical Reasons to Forego Remote Blood Substitution

There can be cryonics scenarios that warrant skipping remote blood substitution although the expertise and equipment is available. For example, the cryonics standby team can significantly reduce transport time by preparing the patient for cold transport instead of adding the extra step of blood substitution. In such cases the standby team is confronted with the choice of doing remote blood substitution at the expense of catching the next airplane for shipping the patient. In case there are regular flights, this can be a difficult trade-off, but in case the next flight is not scheduled for, let us say, the next 48 hours, it would be more prudent to give priority to getting the patient to the cryonics facility for cryoprotectant perfusion promptly. Another example is when the cryonics organization has been able to find a nearby cooperating funeral home for preparation of the patient but blood substitution is not possible or is not permitted at the facilities. In such cases, the standby team has to either forgo blood substitution or expose the patient to longer cardiopulmonary support transport times during transport to another, more distant, funeral home. Such a scenario occurred with Cryonics Institute Patient #81.

Technical Reasons to Forego Remote Blood Substitution

Perhaps with the exception of closed circuit cryoprotective perfusion, remote blood washout is one of the most challenging interventions in cryonics. Remote blood substitution requires two distinct medical skills: surgery and perfusion. Although cryonics organizations are often able to persuade funeral directors to use their professional skills to obtain access and cannulate the vessels, the lack of people with extensive knowledge about extracorporeal perfusion, let alone documented experience and skills, presents a major challenge for cryonics organizations. Unlike other interventions, such as placing an intravenous (or intraosseous) catheter or external cooling, extracorporeal perfusion of the patient can have serious negative consequences for the patient if mistakes are made. Examples of such mistakes

include the pumping of air into the patient, excessive perfusion pressure (vessel damage), and incorrect connection of the perfusion circuit to the patient's vessels (such as connecting the arterial side of the perfusion circuit to the venous side of the patient). The case report of Cryonics Institute Patient #81 generated a lot of discussion about what the necessary technical and professional requirements should be to operate the air transportable perfusion circuit (ATP), and there is a growing consensus that increased effort should be made to secure the assistance of professional perfusionists and facilitate more extensive training for existing cryonics team members. In the absence of people who have demonstrated skills to do perfusion, a decision to do remote blood washout for the patient should not be made lightly. For this reason, since 2011 Alcor has had a policy using the contractor Suspended Animation, Inc., (SAI) to perform blood substitution of Alcor patients in the continental United States outside of Arizona.SAI in turn contracts with cardiothoracic surgeons and clinical perfusionists, who are medical experts in accessing large blood vessels and diluting or replacing blood.

Errors in Perfusate Composition

Although the composition of MHP-2 has been defined and stable through most of the 21st century, there have been historical inconsistencies.One of the most problematic has been anecdotal claims that during the 1980s MHP-2 was originally prepared by mixing ingredients in defined proportions, but not defined absolute concentrations. Final absolute concentrations were the result of titrating the ingredient mixture with water to achieve a measured target tonicity of approximately 400 mOsm, with a substantial portion of this tonicity due to undefined NaCl content of the HES powder then used. MHP-2 is currently prepared according to defined absolute amounts of each ingredient. If made correctly, it will have a measured tonicity using a freezing point osmometer of approximately 320 mOsm. Instead of an endpoint that is always achieved, tonicity is now a quality control check for stoichiometry errors.

Errors in circulating formulas of MHP-2 and mistakes made during perfusate preparation can also lead to adverse effects during blood substitution, ranging from slight diversions from optimal perfusate composition to major consequences such as decreased viability and increased

edema. Mistakes that have been made in perfusate preparation in cryonics include elimination of sodium bicarbonate (under the assumption that the change from MHP to MHP-2 was to substitute HEPES for sodium bicarbonate), errors in stoichiometry in case of components that are supplied in hydrated versions, incorrectly low concentrations of calcium-chloride, and preparation of the solution with physiological pH (7.4) instead of the higher pH range that (8.0-8.2).

Some formulations of MHP-2 include insulin in the same amount as Viaspan (40 I.U. per liter). The question of whether to add insulin to MHP-2 remains unresolved. Some cryonics advisors believe that there is no evidence that this component is essential for asanguineous hypothermic resuscitation, and may even be detrimental. Other cryonics advisors believe that it may be essential to support non-neural glucose metabolism (particularly in the 5 to 15 degrees Celsius range) and to prevent rigor mortis in heart and skeleton muscles by facilitating glucose entry through the GLUT4 transporter.

MHP-2 includes the antioxidant tripeptide glutathione to scavenge free radicals. Because glutathione oxidizes during prolonged storage, it is generally agreed that it would be better to add glutathione at the last minute during a case. Before this can be accomplished a number of challenges need to be overcome: the glutathione needs to be kept chilled during transport, a practical method of introducing it to the solution must be found, an undesirable drop in final pH (gluthathione is acidic) needs to be avoided, and addition of the glutathione in the field should not increase osmolality excessively. Similarly, concerns have also been raised about long term degradation of heparin in MHP2. Although it has occasionally been advised that polypeptides like glutathione and glycosaminoglycans like heparin should be added during use, current practice in cryonics has been to add them during perfusate preparation.

Because the challenges discussed here can be overcome by improvements in perfusate preparation and better quality controls, these problems do not reflect deficiencies in the formulation and use of the perfusate itself. This still leaves the question of whether remote blood washout can aggravate injury in ischemic patients unanswered. Dr. Southard, one of the inventors of Viaspan (UW solution), discussed similar concerns in a recent interview about the future of organ preservation solutions:

> In clinical organ preservation/transplantation, there are many unexplained incidents of reperfusion injury. This is characterized by delayed graft function in the liver and kidney. We do not see this in our animal models. Thus, there are some differences between how experimental animals and human donor organs respond to organ preservation. The difference may be related to the fact that the UW solution was developed to preserve the "ideal organ." This is one taken from a relatively young and healthy lab animal donor and transplanted into a healthy recipient. In the clinics, the donors are usually brain-dead (brain trauma), remain in the ICU for periods up to a day or more, are treated for hypotension, and come from an uncontrolled group of donors. Therefore, we are now studying how UW solution preserves organs from the "less-than-ideal" donor. We are simulating the clinical condition by inducing warm ischemia or brain death in experimental animals to determine if UW solution is suitable for these types of organs. If not, we will develop an ideal method to preserve these less-than-ideal donor organs.[27]

Reperfusion injury

The No-Reflow Phenomenon

In a landmark 1968 study[28], Ames III et al. introduced the phenomenon of "no-reflow," the observation of impaired blood flow after ischemia. The no-reflow phenomenon is treated separately from reperfusion injury for two reasons. The most important reason is that although cerebral ischemia-induced "no-reflow" may reflect brain injury, impaired flow itself does not constitute injury and can be (partially) reversed by specific interventions such as hypertension and hemodilution. The other reason is that most explanations that have been put forward for the no-reflow phenomenon such as post-ischemic hypotension, increased blood viscosity, blood coagulation, plugging of vessels by inflammatory blood components, and cell swelling, can all be mitigated by washing out the blood with an hyper-osmolar organ preservation solution and increased perfusion pressure. Instead of contributing to or aggravating post-ischemic no-reflow, remote extracorporeal perfusion seems to be the ideal intervention to reverse no-reflow and restore flow to regions of the brain that had been previously closed off by ischemia. The ability to vastly

improve cooling rates during extracorporeal perfusion can also delay ischemic depolarization and ischemia-induced cell swelling.

There are two ways remote blood substitution *may* produce adverse effects similar to no-reflow. If the patient has been in circulatory arrest for an extended period, reintroduction of oxygen may introduce free radical injury to cell membranes, and consequently, impaired perfusion. To the extent that there is merit to this scenario this might be avoided by "anoxic blood substitution" and inclusion of antioxidants and free radical scavengers in the organ preservation solution. The second way in which blood substitution may produce adverse effects similar to no-reflow is that if the composition of the organ preservation solution itself is of such a nature that some components will contribute to edema and impaired flow. This is especially a concern in case when edema would *exceed* what we would expect with leaving the blood in the patient during cold transport. This issue is not intrinsic to blood substitution as such but raises important challenges in terms of perfusate formulation. We will address this concern in more detail when we discuss potential improvements to perfusate composition for use in prolonged static use.

The reason to focus on reperfusion injury *induced* by blood substitution instead of ischemic injury resulting from cold circulatory arrest is because the ischemic injury that one would expect during prolonged periods of asanguineous circulatory arrest should occur equally or even more so during cold circulatory arrest in patients whose blood has not been washed out. Moreover, it is the very ability to manipulate the composition of the intracellular and extracellular environment in asanguineous cold circulatory arrest that permits longer maintenance of viability. Therefore, it is more instructive to look for instances where *the process of blood substitution itself* may produce or aggravate ischemic injury.

Free Radical Injury

There are least three scenarios in cryonics in which blood substitution could produce or aggravate free radical injury:

1. Prolonged circulatory arrest without stabilization

2. Trickle flow perfusion during cardiopulmonary support

3. Intermittent perfusion during surgery prior to blood substitution

In the first scenario, the patient has suffered a long period of normothermic ischemia before or after pronouncement of legal death. Although there has never been a rigorous attempt to quantify the maximum period of warm ischemia that a cryonics patient can be exposed to beyond which restarting circulation does more harm than good, it is usually recommended not to oxygenate the patient during cardiopulmonary support after 30 minutes of circulatory arrest. When a patient has been without circulation for a long period, remote blood substitution is sometimes forgone altogether. In case circulatory arrest is immediately followed by blood substitution, free radical injury can take two forms: 1) when the perfusate is not oxygenated, dissolved oxygen in the perfusate and remaining oxygen in the patient's vessels can cause free radical-mediated reperfusion injury, and 2) when the perfusate is oxygenated, additional oxygen will be in introduced to the patient and may cause additional free radical injury if the oxygen cannot be metabolized before contributing to free radical formation.

It should be noted that the first scenario cannot be completely avoided. Even if the perfusate itself would not contain any dissolved oxygen, some residual oxygen in the vessels of the patient would be introduced to the ischemic brain. This would be even be the case if the patient were perfused with an inert gas because the initial perfusate that enters the patient will move the remaining oxygenated blood to the brain. Since generation of harmful reactive oxygen (and nitrogen) species is virtually instantaneous upon reperfusion it is doubtful if such initial events of reperfusion injury can be completely avoided during restoration of circulation in ischemic patients. The question therefore is whether such reperfusion injury is worse than leaving the blood in the patient and accepting slower cooling rates. It cannot be argued that circulation is going to be restored during cryoprotective perfusion at any rate, so remote blood substitution will only produce this injury at an earlier stage. For starters, when blood substitution is being eliminated the patient will typically be shipped at water ice temperature. As a result, cryoprotective perfusion will start around the freezing point of water, which may reduce free

radical injury. Further, if remote blood substitution is performed, the patient will go through two separate cycles of circulatory arrest and reperfusion. Reperfusion injury caused during the first cycle of perfusion may worsen the prospects for subsequent perfusion with a vitrification agent as a result of increased edema and cell membrane damage. On the other hand, by removing the blood, blood components that can contribute to impairment of cryoprotectant perfusion (such as platelets, red blood cells, and white blood cells) will be removed from the circulatory system of the patient. Although it is possible to outline the potential advantages and disadvantages of remote blood washout in patients with long periods of circulatory arrest, it will not be possible to make any specific, let alone quantitative recommendations unless such cryonics scenarios are investigated in a relevant research model (see below)

The second scenario in which reperfusion injury may present a problem is when a patient undergoes prolonged periods of low-flow cardiopulmonary support. The assumption is that cerebral blood flow will not be sufficient to meet cerebral oxygen demand but will be adequate to cause free-radical medicated injury. There are three arguments against this scenario. The first is that such low-flow states are better than no flow at all. Steen et al. found that dogs could sustain only 8 to 9 minutes of complete ischemia but 10 to 12 minutes of incomplete ischemia (cerebral blood flow less than 10% of control) without neurological impairment[29]. The second is that instead of being adverse, such low flow states may mitigate reperfusion injury because they “stabilize” ischemic injury that occurred during circulatory arrest and simultaneously reduce reperfusion injury during full restoration of circulation by cardiopulmonary bypass through post-ischemic conditioning and up-regulating antioxidant defenses[30]. The third is that prolonged low flow perfusion will induce hypothermia (albeit less rapidly than would have been possible during aggressive cardiopulmonary support). Because decreased body temperatures will mitigate ischemic injury (and thus free radical damage), at some point the combination of low flow blood flow and hypothermia may eliminate ischemic injury.

Since there can be enormous variability in the quality of treatment during both short and prolonged periods of cardiopulmonary support, it is difficult to

make any firm recommendations about which events during cardiopulmonary support are indications, and which are contra-indications, for blood substitution. It can be argued, however, that because cryonics stabilization medications contain a number of antioxidants and free radical scavengers, there appears to be less risk of inducing major reperfusion injury by following cardiopulmonary support with cardiopulmonary bypass, especially when the patient is at low temperatures and periods of halted blood flow will be minimized during surgical preparation for blood substitution. If additional ischemic injury is incurred during cardiopulmonary support, this scenario resembles the first scenario in which blood substitution is performed in a patient with prolonged circulatory arrest, although we would expect different mechanisms of injury during prolonged cardiopulmonary support such as pulmonary edema and increased free radical damage. So far the relationship between various forms and durations of cardiopulmonary support and its effects on subsequent blood substitution has not been the subject of focused research in cryonics.

The third scenario is one in which long interruptions or intermittent interruptions in chest compressions during surgery increases reperfusion injury during blood substitution. This concern is more relevant than it was in the earlier days of cryonics when focused attempts were made to minimize interruptions of cardiopulmonary support during surgery. It is not a-priori clear whether complete interruption of chest compressions during surgery or intermittent interruptions are more harmful. The latter scenario may be more harmful because it allows for continuous cycles of free radical generation. Although cardiopulmonary bypass can induce more rapid cooling than external cooling, it seems not advisable to do surgery at near normothermic temperatures. Such a scenario would mimic a clinical situation in which circulatory arrest would be produced at mild hypothermic temperatures. When more advanced cooling techniques like cold cyclic lung lavage (liquid ventilation) will become available in cryonics, the option of delaying surgery until a “safe” temperature is reached should come within reach. Ischemic injury and reperfusion injury induced by interruptions in circulation during surgery will rarely be an argument to forgo blood substitution because the decision the do surgery reflects a decision to wash out the patient’s blood.

Only during exceptional circumstances may observations made during surgery present a reason to terminate further attempts at blood washout. The most obvious example is when the surgeon believes the patient's circulatory system is in such a poor state that it should not be further damaged until cryoprotective perfusion in the cryonics facility.

Calcium Overload

Reperfusion injury is not confined to reoxygenation injury. One of the major mechanisms of injury during cerebral ischemia is calcium-induced cell death. Intracellular calcium overload is produced by a number of mechanisms including excitotoxicity, inhibition of ATP driven membrane pumps, and generation of free radicals. Intracellular calcium overload can be aggravated by restoring circulation, either through closed chest cardiopulmonary support or cardiopulmonary bypass. As such, the opportunities for increased calcium-induced injury are similar to those for free radical injury. Even when the stabilization medications protocol includes agents that chelate calcium such as citrate or EDTA, restoration of circulation can still contribute to delivering extra calcium to ischemic cells during the early stages of perfusion. The analogy between free radical injury and calcium overload induced reperfusion injury is not complete. Whereas the patient remains at risk of free radical induced injury during all parts of cryonics procedures, increasing calcium overload is only a major risk during cardiopulmonary support when calcium has not been chelated by citrate. Cardiopulmonary bypass also allows for manipulation of the patient's extracellular and intracellular environment which can be used to reduce calcium induced cell injury.

Vascular Injury

Perhaps the biggest concern about following prolonged periods of cardiopulmonary support with cardiopulmonary bypass is that this introduces another opportunity to produce or aggravate injury to (micro)vessels. As discussed earlier, such injury can be produced by inexperienced persons running perfusion equipment. This can be avoided by employing professional stabilization teams and skilled perfusionists. But it is also conceivable that cardiopulmonary bypass will invariably worsen the state of the circulatory

system in patients with (advanced) ischemic injury. Such injury can be produced by free radicals, calcium overload and other mechanisms such as neutrophils and platelets (or a combination of those factors) and aggravated by restoring circulation at high temperatures. This last possibility highlights the reason why this concern cannot be dismissed by stating that a patient needs to be perfused with a cryoprotective agent requires circulation at any rate. First, the temperatures at which patients are typically perfused with a cryoprotective agent are lower than those at which remote blood substitution is initiated. As such, biochemical reactions that can worsen vascular injury happen at a faster pace during blood washout. Second, total perfusion times for patients whose blood is replaced with an organ preservation solution is longer because a) the organ preservation solution needs to be washed out again, and b) during blood substitution, "closed circuit" recirculation of the perfusate is often necessary to approach the freezing point of water prior to air transport of the patient. Although edema produced during blood substitution may be reversed during cryoprotective perfusion by circulating a hyper-osmotic / hyper-tonic perfusate, such measures increase cryoprotective perfusion times and risk additional injury to cells and the blood brain barrier as a result of the alternating osmotic fluid cycles between cells and the patient's vessels.

Although there are a number of clinical arguments to replace the patient's blood with an organ preservation solution for transport at ultra-profound temperatures, we know that blood itself is not harmful to (non-ischemic) vascular cells. How ischemic vessels respond to long storage times with an a-cellular asanguineous perfusate is still mostly unexplored territory. As will be discussed in the section about improvements to perfusate composition below, vascular injury during perfusion may be mitigated by including components that can "seal" damaged membranes, allowing for longer perfusion and transport times without edema.

Contra-Indications for Remote Blood Substitution

The lack of validation of blood substitution in models that reflect the typical cryonics transport situation makes it difficult to formulate a set of hard indications and contra-indications for remote blood substitution.

The **indications** for remote blood substitution are roughly equivalent to what constitutes a good cryonics case: patients who received prompt intervention after pronouncement of legal death, aggressive cardiopulmonary support, and minimum interruptions of circulation prior to blood washout resulting in reduced ischemic injury and rapid cooling. In qualitative terms, in such cases, near-physiological circulation is achieved after cardiac arrest and cardiopulmonary bypass will constitute a continuum of this.

The **contra-indications** for remote blood substitution range from "pre-mortem" patient pathologies to practical and logistical challenges. What follows is a list of contra-indications derived from the discussion above.

- Omitting remote blood substitution will significantly reduce transport time
- The nearest funeral home that allows blood substitution will result in excessive cardiopulmonary support times
- No team members with extensive experience and knowledge of cardiopulmonary bypass are present on the case
- Inspection of the blood organ preservation solution finds bacterial growth
- Inspection of the blood organ preservation solution composition finds serious errors in perfusate composition
- Observations of systemic edema during cardiopulmonary support
- Active gastrointestinal bleeding at the time of cardiac arrest
- Prolonged splanchnic ischemia or severe abdominal swelling
- Severe pulmonary edema
- Severe cerebral edema
- Prolonged periods of warm cerebral ischemia (>60 minutes)

As the last contra-indication indicates, sometimes there may be arguments to forgo blood substitution when the risks may not be visible yet. In cases of prolonged warm circulatory arrest, initiating cardiopulmonary bypass may produce fulminating cerebral edema. As a result, the only opportunity to perfuse the brain will be "used up" during blood substitution instead cryoprotective perfusion.

Improved Organ Preservation Solutions for Cryonics

As experimental studies of static storage of brains and clinical limitations of static storage of organs with similar tolerance to ATP deprivation (such as the heart) indicate, it is not likely that remote blood substitution can secure viability of the brain during typical cryonics transport times, often exceeding 24 hours. This does not mean that remote blood substitution is contra-indicated for long transport times because there are a number of other advantages to remote blood substitution. Unless fundamental breakthroughs in hypothermic organ preservation solution design are made that allow substantial extension of cold hypothermic storage of the brain, more emphasis on designing solutions that prevent development of edema during transport seems to be the most promising research direction. Another interesting research area is to use some of the findings of the normothermic resuscitation experiment for improved composition of organ preservation solutions. An obvious example would be to include neuroprotective additives to the perfusate to extend tolerance to cold ischemia. This direction is further warranted by the fact that during remote blood substitution most of neuroprotective medications are flushed out.

Protecting Against Hypothermia and Ischemia-Induced Edema

The major concern that has been raised about MHP-2 is that blood substitution may have the unintended result of increasing edema during cold storage, instead of decreasing it. Reasons why this could happen have been discussed earlier in the text: lack of hyper-osmolality (or even hypo-osmolality) or cardiopulmonary bypass in (very) ischemic patients. Another reason may be that some of the "impermeants" like glucose and mannitol will cross cell

membranes and increase intracellular water accumulation. One modification in MHP-2, especially in light of its use during prolonged transport times, would be to substitute higher molecular weight impermeants like raffinose (594 mW) and lactobionic acid (mW) for glucose and mannitol. The new organ preservation solution by 21st Century Medicine (21CM) called TransSend contains the impermeants polyglycerol (which is the ice blocker Z-1000 used in 21CM vitrification solutions) and alpha-lactose.

Colloids

The importance of the colloid hydroxyethyl starch in MHP-2 needs to be questioned in light of the fact that MHP-2 is almost invariably used for long cold transport instead of continuous low flow hypothermic perfusion. Concerns have been raised about HES effects on the viscosity of solutions and red blood cell (RBC) aggregation. Recent research into cold organ preservation indicates that the initial washout of organs can be optimized by using an HES-free solution with a non-RBC-aggregating colloid as the first step. Current cryonics protocols do not include such a two-step approach. Newer cold organ preservation solutions from 21st Century Medicine such as TransSend do not contain HES at all. An alternative for HES may be polyethylene glycol (PEG). Improved results have been found in solutions in which PEG is substituted for HES. Another advantage that PEG may have over other colloids is its ability to “seal” injured membranes, allowing for reduced cell damage and improved cryoprotective perfusion. Another membrane sealing polymer is the large molecular weight tri-block polymer Poloxamer 188 (P188). P188 is effective in much lower concentrations than PEG and has been shown to protect hippocampal neurons against neurotoxin-induced cell membrane damage[31] .

Energy Substrates

MHP-2 includes adenine and D-ribose to assist regeneration of ATP during reperfusion. Because organ preservation solutions in cryonics are not used with the objective of near-term resuscitation, it is not evident that these components are of value during cold transport of the patient. On the other hand, providing energy substrates during cold storage may support the

remaining cellular metabolism during hypothermic transport. Such components may be especially important if the solution is not used as a static solution but for continuous or low-flow perfusion. Although UW solution does not include any glucose at all, low amounts of glucose have been maintained in MHP-2 to support anaerobic respiration and inhibit rigor during cold ischemia. Because of concerns about glucose-induced cell swelling and production of lactate accumulation, an alternative would be to substitute an alternative energy substrate for glucose.

Neuroprotective Agents

Theoretical considerations would predict that the inclusion of neuroprotective agents in organ preservation solutions should be able to extend the period that the brain can tolerate cold ischemic exposure. The ability to resuscitate dogs from 3 hours of ultraprofound hypothermic circulatory arrest without blood substitution would suggest that these times can be extended if organ preservation solutions are designed to mitigate cerebral ischemia. Such solutions will not only include components to reduce hypothermia-induced cell swelling and acidosis but also agents to intervene in various parts of the ischemic cascade. Because warm ischemia is not equivalent to cold ischemia, it cannot be assumed that just adding all (or most) of the compounds that enable resuscitation from ~16 minutes of cardiac arrest will produce a comparable increase in preservation time during ultraprofound hypothermia, although such compounds should have priority in screening different formulations of organ preservation solutions. Some candidates for inclusion in improved organ preservation solutions are Tempol, FK-506 (Tacrolimus), Na+/H+ exchangers inhibitors, and increased magnesium concentrations.

Research into Remote Blood Substitution in Cryonics

Although blood substitution is routine in remote cryonics cases (or should be routine), the only documented evidence for its efficacy was done in the 1980s by Leaf and Darwin, and some pilot experiments by Leaf in 1970s. These experiments demonstrated that dogs could be resuscitated from up to 5 hours of low flow asanguineous ultra-profound hypothermia. Other experiments

with MHP (or variants thereof) have been done, but these experiments have not been (formally) documented. As a consequence, most of the rationale for using such organ preservation solutions has been based on extrapolation and theoretical considerations.

In 2005, Yuri Pichugin at the Cryonics Institute used the K+/Na+ ratio assay to investigate viability of rat brain slices after various durations of warm and/or cold ischemia. He found that brain slice viability was 43% for 24 hours of cold ischemia, 0% for 48 hours of cold ischemia, 63% for 10 minutes of warm ischemia plus 6 hours of cold ischemia, and 32% for 1 hour of warm ischemia plus 23 hours of cold ischemia[32]. The author also reports that he did not find any improvements in brain viability when whole rat brains were perfused with "Viaspan (University of Wisconsin organ preservation solution), RPS−2(Renal Preservation Solution) and its modifications, MHP−2 (Mannitol - Hydroxyethyl starch – Perfusion solution; M. Darwin and et al, Alcor), Renasol H (Renal Solution with Hydroxyethyl starch; Dr. Fahy and et al, 21 CM), and New Organ Preservation Solution of Kyoto University (Chen F. and et al, Japan, 2004)," prior to 12 or 24 hours of cold storage at 4 degrees Celsius. Neither did any other compound used to prolong cold storage times offered great promise. In 2006 and 2007, Pichugin experimented with dehydration, inert fluids with silica gel, and non-toxic concentrations of aldehydes to stabilize cell membranes to improve viability during cold storage but did not observe encouraging results. He did report "stable vitrification" for sheep brains that were perfused after 24 hours of cold ischemia. These results seems to be at odds with the profound ultrastructural damage Mike Darwin, Jerry Leaf and Hugh Hixon (1982-1983) observed when ischemic cats were perfused with glycerol after 30 minutes of warm ischemia and 24 hours of cold ischemia. The investigators did not investigate viability but histological and ultrastructural changes between non-ischemic and ischemic cats after perfusion with glycerol, freezing and rewarming. Impaired blood washout, cerebral edema, and profound ultrastructural freezing damage was observed in the ischemic animals[33]. It should be noted that the animal model, the nature of ischemic exposure in these experiments (cold vs. warm plus cold), and the cryoprotectant used was not identical to Pichugin's model. Also, the research

by Darwin, Leaf, and Hixon is the only documented research to date that studied the effect of ischemia and cold ischemia on cryoprotective perfusion.

In 2007, Gregory Fahy reported the results of hypothermic brain preservation that was done at 21st Century Medicine. Initially, the researchers found massive ultrastructural damage in brains that had been stored cold for 24 hours. But better results were obtained when perfusion pressure was raised during fixation from 60 mmHg to 120 mmHg, which seem to indicate that the initial results were a fixation artifact. In another series of experiments, 3 hours of static cold storage (which reflects the current record in total body washout) and 5 hours of continuous hypothermic perfusion were compared. K+/Na+ assays on isolated hippocampal slices demonstrated better results for 5 hours of continuous perfusion, with viability scores not dropping for the duration of the experiment. Although continuous perfusion was demonstrated to be superior to cold static storage of brains, this achievement did vary, depending on the composition of the "brain preservation solution." One preservation solution did not produce better results during continuous perfusion over static cold storage. This finding seems to support the view that the formulation of the organ preservation solution can affect cerebral viability during hypothermic perfusion. The researchers also demonstrated improved maintenance of electrical activity in the brain after continuous perfusion, although on this measure of viability, electrical activity dropped during the duration of perfusion, which seems to be consistent with the hypothermic brain preservation experiments of White et al.

Since 2008 Advanced Neural Biosciences has conducted extensive series of rat experiments to investigate the effects of ischemia and blood substitution on perfusion of the brain, cryoprotectant distribution, and ice formation. The most meaningful finding (and corroboration of Alcor's protocol to do remote blood substitution) is that up to at least 48 hours of cold ischemia, blood substitution with an organ preservation allows for improved cryoprotectant perfusion and decreased ice formation.It was found, however, that the composition of the organ preservation solutions is important. There was little difference in case the blood was replaced with sodium chloride, better results were obtained with RPS-2, and the best results were observed in the case of Alcor's MHP-2. The tentative conclusion to be drawn from this is that remote

blood substitution may not be effective in keeping the brain viable for more than 4 hours but that this procedure is still warranted up to at least 48 hours of cold ischemia (without substantial warm ischemia) to improve post-transport cryoprotective perfusion. Despite these beneficial effects researchers at Advanced Neural Biosciences have not been able to identify an organ preservation solution (including newer "brain preservation solutions) that decreases edema during post-transport cryoprotective perfusion.

In another series of experiments Advanced Neural Biosciences has investigated whether conducting blood substitution after various periods of warm ischemia is contra-indicated because it may only introduce additional damage as a result of "reperfusion injury." In these experiments the researchers found, however, that following periods of warm ischemia by blood substitution is always better than not removing the blood prior to cold storage, up to 1 hour of warm ischemia, after which extensive ice formation was observed in both protocols. The tentative conclusions that can be drawn from these preliminary studies is that episodes of warm ischemia do not undermine the case for remote blood substitution but if these episodes are extensive (exceeding 1 hour) this procedure cannot be expected to reverse the negative effects of warm ischemia.

The Future of Blood Substitution and Field Cryoprotection

Even under ideal circumstances, contemporary technologies do not allow functional recovery of the brain from more than 3 hours of circulatory arrest, or 5 hours of low flow perfusion. In addition, energy-depletion during prolonged cold storage contributes to edema. This raises the question whether there are credible alternatives for existing remote blood washout procedures. The two major alternative solutions identified are doing continues perfusion during transport and eliminating remote blood substitution and shipping on water ice for "field cryoprotection."

The advantage of low-flow continuous perfusion would be the ability to keep the brain viable for a longer period of time en-route to the cryonics facility. There are a number of challenges with this approach. The typical transport times of patient that are pronounced legally dead in a remote location

will usually exceed the period in which it is still possible to maintain viability of the brain. As a consequence, this procedure will still fall short of Alcor's goal to secure viability of the brain by contemporary criteria. Another complication is that most Alcor patients will suffer some degree of ischemic injury pre- and/or post-pronouncement. Conducing low-flow continuous (or intermittent) perfusion on such patient can produce increasing (whole body) edema en-route to the facility. This is not just a detrimental development by itself but can also limit meaningful cryoprotective perfusion the operating room. Another complication that needs to be mentioned here is logistical. Even more than "conventional" static remote blood substitution, continuous perfusion of the patient requires the use of designated vehicle that is equipped to support the patient all the way from the start of washout until arrival at the facility. This would also require the non-stop presence of (professional) perfusionists.

A more realistic alternative that can both satisfy the aim of keeping the brain viable by contemporary criteria and prevent the edema that is typically associated with remote blood washout (and even continuous perfusion) is "field cryoprotection." In field cryoprotection the remote blood washout procedure is not followed by packing the patient in ice for shipping but cryoprotective perfusion and shipping on dry ice. One major advantage of this procedure is that most circuits that are being used for remote blood washout can also be used for step-wise cryoprotective perfusion. The topic of field cryoprotection will be briefly discussed again in the following section on the conduct of blood washout and will be more extensively treated in the chapter on cryoprotective perfusion.

Part 2

Extracorporeal Perfusion in Cryonics

While it can be argued that the practice of cryonics in the early days "straddled cryobiology and mortuary practice" the possibility (and desirability) of using mainstream medical extracorporeal technologies was already recognized by Robert Ettinger in his seminal cryonics book "The Prospect of Immortality." He writes, "With fully adequate preparation, equipment, and personnel, the cooling phase seems to present little problem in most cases. Heartlung machines and heat exchangers are available at many hospitals. The cardiopulmonary bypass technique is commonly used for open-heart surgery, with cooling of the blood and body from the normal of about 38°C down to 20°C, and sometimes lower; this technique has been described, for example, by Sealy and co-workers. Apparently it could also be used, depending on the cause of death and opportunity for preparation, to cool freshly dead bodies quickly and safely, with no damage to the brain." Following this observation, between 1966 and 1967 Dr. Dante Brunol wrote a protocol for the cryopreservation of cryonics patients that included closed chest compressions, the use of extracorporeal technology for cooling, blood washout, and cryoprotective perfusion.

Until at least the early seventies, however, the utilization of mainstream medical procedures and extracorporeal technologies for cooling, blood washout, and cryoprotective perfusion was not well developed and most cases were either conducted in funeral homes with (crude) embalmer's equipment or cryonics patients just received a "straight freeze."

This situation changed when Fred and Linda Chamberlain recognized the desirability of using mainstream extracorporeal technologies for cryonics patients and collaborated with Michael G. Darwin on a perfusion machine for cryonics patients. At the end of the 1970's thoracic research surgeon Jerry Leaf entered the field and extracorporeal medical technology was incorporated

in cryonics protocols and adapted for use in cryonics patients. Despite (sometimes turbulent) changes in the field of cryonics, medical extracorporeal technologies have been remained a part of Alcor's procedures until the present day.

The use of extracorporeal technologies in cryonics is three-fold. It is used for remote blood washout, for cryoprotective perfusion, and for cryonics-associated research. In this section we will confine ourselves to the use of extracorporeal technologies for total body washout. Some research results obtained with extracorporeal technologies have been discussed in part I of this section and the use of extracorporeal technologies in cryoprotective perfusion will be discussed in the next section. While bypass technologies can potentially be used instead of closed chest compressions during the early stages of stabilization to improve cerebral perfusion and cooling, to our knowledge, emergency bypass resuscitation technologies have never been used in a cryonics case to date, despite their theoretical desirability.

Our treatment of the use of extracorporeal technologies will review the four major approaches that have been or are utilized in cryonics, starting with the least advanced approach. In short we will review: (1) open circuit gravity-assisted blood washout (2) blood washout with embalmers equipment (3) in-house fabricated air-transportable perfusion circuits and (4) utilization of professional extracorporeal perfusion equipment and contract perfusionists. We will follow this review with an exposition of specific issues associated with the utilization of extracorporeal technologies in cryonics and areas of debate that have emerged over the years. We conclude this section by identifying future potential directions for the use of extracorporeal technologies in cryonics.

Surgery

All methods of blood substitution require access to the circulatory system of the patient and the placement of cannulae to flush out the blood and replace it with an organ preservation solution. While there are a variety of locations to place the perfusion cannulae, the most common practice in cryonics has been to cannulate the femoral vessels.Other options include cannulating the heart vessels (as in mainstream cardiopulmonary bypass) or cannulating the neck

vessels. In some cases these options may be pursued when the femoral vessels are too compromised to cannulate or when local edema makes femoral cannulation not feasible.

Conducting surgery for blood washout is not a basic cryonics procedure suitable for volunteers and is usually done by either professional surgeons or staff members extensively trained in the procedure. In some occasions the mortician can be persuaded to conduct surgery for blood washout but it is important to recognize that morticians are not trained do such procedures with recovery as an endpoint and may only be able to offer limited skillset and supplies. In addition, surgery for blood washout involves making incisions and dissecting tissue; as such, this procedure should be performed in such a manner as to protect the team members from infection by wearing gloves, face masks, hair covers etc. In case of serious infectious diseases (such as HIV), extraordinary precaution need to be taken in consultation with Alcor's medical advisors.

Gravity-Assisted Blood Substitution

The most basic method of washing out the patient's blood and replacing it with an organ preservation solution is to rely on gravity instead of pumps. Although it is possible in theory to use this approach in conjunction with advanced surgical skills and placement of various arterial and venous cannulae and monitoring equipment, a "gravity flush" of this kind is usually done with the assistance of a mortician or trained staff member. The absolute minimum in terms of surgical skill is the ability to place a (large bore) cannula in a major artery (such as the femoral artery) while making a large nick in the corresponding vein for venous effluent.

The pressure to flush the blood from the patient's circulatory system is created by hanging the bag(s) with the blood washout solution in the air. There are a variety of ways of doing this but one easy approach is to use a medical IV pole (which is included with some ice baths) While this method does not give staff members precise and flexible control over flow rate, the pressure in the lines and patient is set by choosing a specific height to hang the bags and the flow rate will be a function of that pressure (and the resistance presented by the cannula and vessels). One advantage of this method is that sudden,

unexpected, high pressure spikes cannot occur. A drawback of this method is that it may sometimes be challenging to achieve the pressures and/or flowrates that a specific case requires. Another drawback is temperature control. While the bags should be stored in ice water prior to deployment, as soon as the bags are hang on a pole the solution temperatures will gradually rise towards room temperature. This will decrease the cooling rate, either necessitating the use of more bags or the need for additional external cooling upon completion of the procedure.

Circuit design in a gravity flush is also basic and can but does not need to include a venous line. Other options are a pressure gauge, a sample port, and a reservoir. As a general rule, a gravity flush is expected to be conducted "open circuit," which eliminates the option to circulate the chilled organ preservation solution to accelerate cooling to 0 degrees Celsius. While the introduction of more complex, pump-operated perfusion circuits or the use of professional perfusion equipment has largely eliminated this method of blood washout, a similar set-up has made a comeback as the default method for "field cryoprotection" (see Section 17).

The image in Figure 16-1 is reproduced from Tanya Jones's 1997 Stabilization and Transport manual.

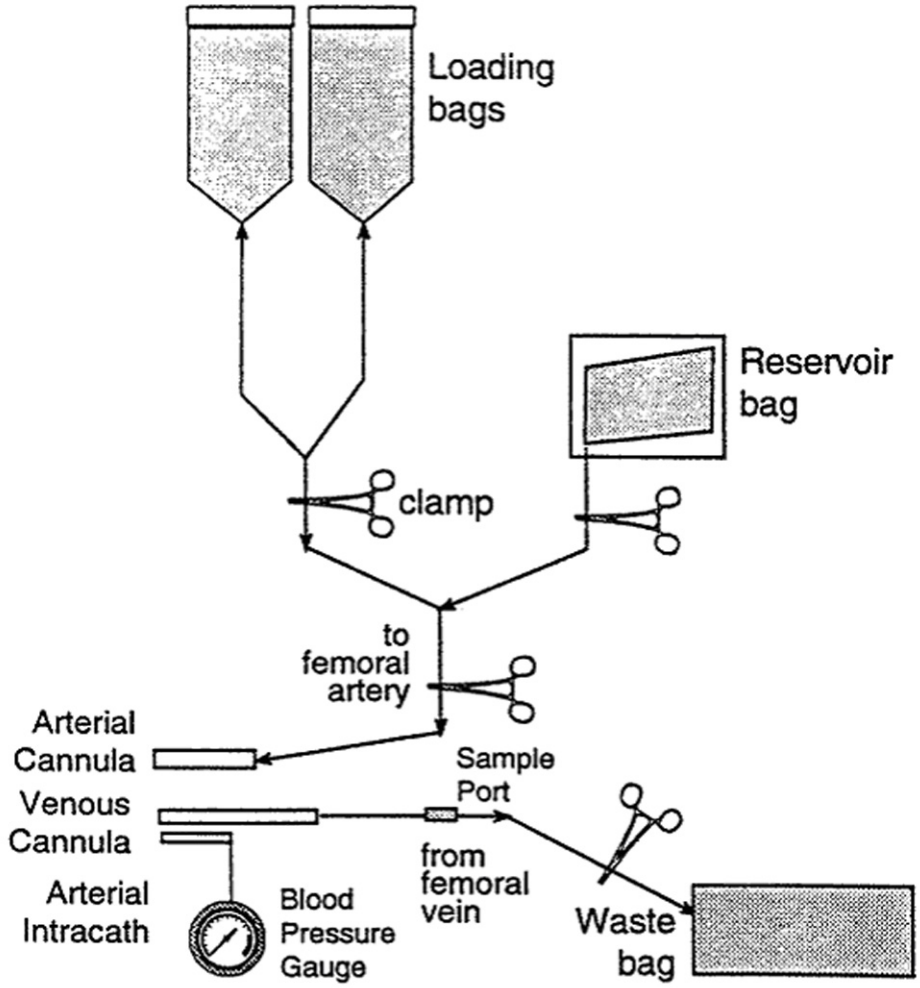

Figure 16-1. An early system of gravity-assisted blood substitution.

Blood Substitution with Embalmer's Equipment

The use of embalmer's equipment can refer to either the use of embalmer's cannulae or pumps. Embalmer's cannulae are often made of steel and come in a variety of models and sizes. An example is shown in Figure 16-2. If the use of an embalmer's cannula cannot be avoided it is important to choose the right type of cannula for the right vessel and make sure to follow proper protocol to prevent air bubbles being introduced in the circuit.

Figure 16-2. An embalmer's cannula.

Embalmer's pumps are rather basic pumps that do not need to meet the more delicate requirements for conducting extracorporeal perfusion in humans. In particular, the finer (quantitative) control over flow rates and pressure is often lacking, both in the pumps and in the circuits used. On the positive side, the processes of embalming and blood substitution share a fundamental objective: the replacement of the patient's blood with another substance. Morticians also have extensive experience with issues such as perfusing a patient with blood clots and edema, so the presence of these phenomena in many cryonics cases should be familiar to them.

The most important reason why embalming pumps should be avoided is because of the risk of introducing particles that can block vessels. As a general rule, embalming pumps and tubing are not consistently sanitized, let alone, sterile. While the elimination of excessive bacterial activity is one aim of embalming, the complete elimination of any particles or harmful substances is not an important objective for them. Using embalmer's equipment can subject the patient to the introduction of particles that can block (micro) vessels, which can compromise subsequent cryoprotective perfusion.If an embalmer's pump is used for blood washout it is extremely important to thoroughly sanitize and clean the equipment (of fixatives!) before use. Great care should also be taken to avoid introducing air in the lines from the rotors in the device that are used to stir the embalmer's solution.

An embalming pump is shown in Figure 16-3. Note that morticians may refer to this as an embalming machine.

Figure 16-3. Embalming pump, also known as an embalming machine.

When utilizing the assistance of a mortician it is important to properly convey the objectives of a blood washout because many funeral directors do not realize that after blood washout and shipment of the patient to the cryonics facility the patient will be subjected to *another* surgical procedure for cryoprotective perfusion.This is one reason why it is not recommended to use vessels such as the carotid arteries and veins. This is particularly important in the case of whole body patients, who are usually cannulated through a median

sternotomy and perfused through the heart. Compromising or nicking the vessels to the brain can greatly complicate perfusion for such patients.

Air Transportable Perfusion Circuit

The Alcor ATP (Air Transportable Perfusion kit) was developed in the 1990s. It constituted a key development in Alcor's remote stabilization capabilities by making its washout protocol available for all members (including international members) through the fabrication of a compact, air transportable perfusion circuit.

The ATP consists of a sterile perfusion circuit with filter and heat exchanger / oxygenator, a reservoir, and a (roller) pump that are contained in a hard shell Pelican case. As a general rule, the ATP was shipped with an additional container with 20 liters of washout solution (MHP-2) and ancillary equipment to set and run the device. An important property of the Alcor ATP is that is designed to run close circuit. This setup reduces perfusate volumes and enhances cooling rates.

As can be seen in Figure 16-4, the lid of the ATP case contains the circuit with filter and heat exchanger/oxygenator and the effluent of the patient is returned to the soft-shell reservoir bag that is also part of the lid. The organ preservation solution is drawn from a separate (chilled) bag and cooled by the in-line arterial heat exchanger and filtered before being delivered to the patient. During the washout phase the venous effluent (blood) is discarded until the effluent is consistently clear. At this stage the circuit is "closed" and the perfusate is allowed to recirculate to cool the patient to as close to 0 degrees Celsius as is practical. A diagram showing the tubing circuit of the ATP is shown in Figure 16-5.

A detailed description of the ATP, its components, and setup instructions can be found in the manual that was produced by one of the writers of this manual (Platt). A number of topics specific to the ATP that are not covered in detail in that manual are discussed here.

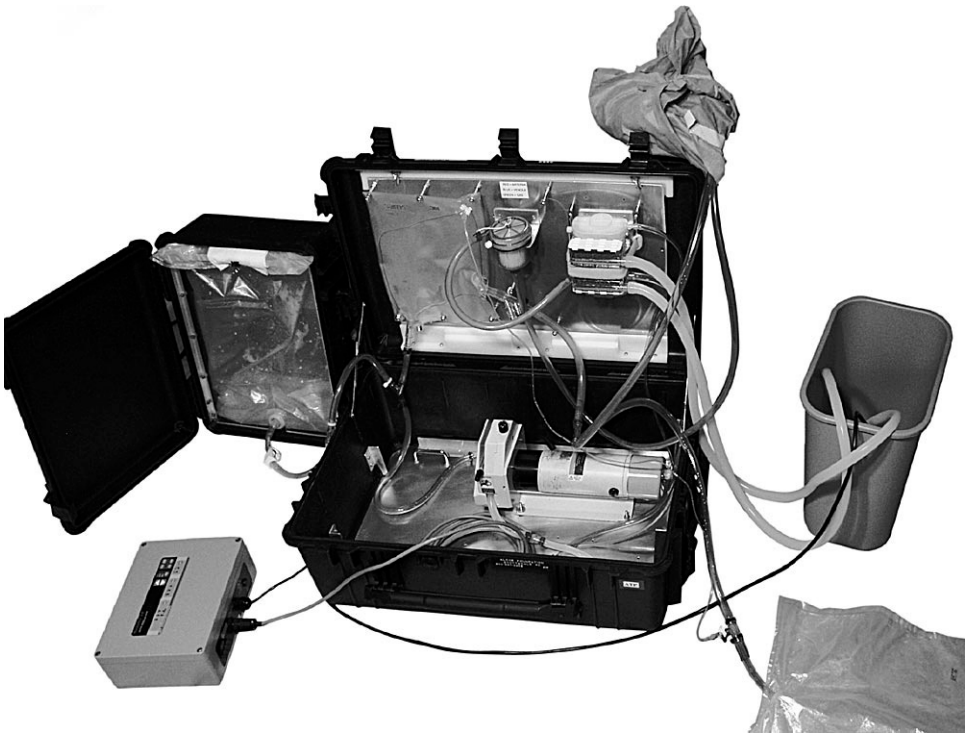

Figure 16-4. The Air Transportable Perfusion kit developed by Alcor for remote blood washout. The fabric bundle at top-right is a sterile pack containing cannulae. The smaller case, open at left, contains perfusate. A large roller pump is visible attached to an aluminum baseplate in the main Pelican container. The box at left is the pump speed controller. A plastic waste basket was often used as an ice-water reservoir, as this did not have to be sterile. A soft reservoir bag is visible mounted in the left side of the open lid of the Pelican container. Beside it is an arterial filter, and to the right of that is a combined oxygenator and heat exchanger. A waste bladder for effluent connection can be seen at lower-right.

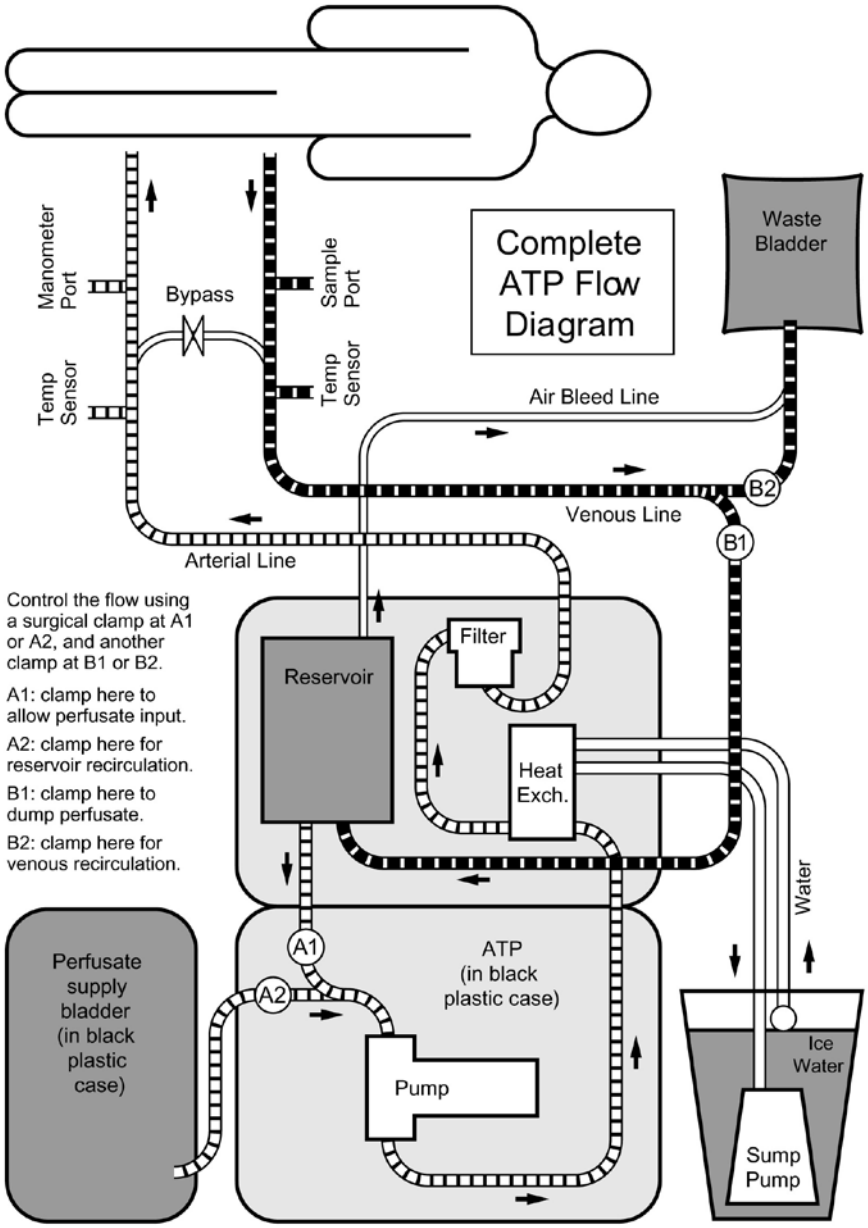

Figure 16-5. Tubing circuit of the ATP.

Most versions of the ATP included an integrated heat exchanger and oxygenator. In practice, the oxygenator has been rarely used in an Alcor case. While this would add another layer of complexity to the operation of the circuit, the omission of oxygenation during blood substitution is not

recommended. If the blood washout is initiated at relatively high temperatures (> 25 degrees Celsius) metabolic demands of the patient may still exceed endogenous energy stores. Oxygenation might even be desirable when blood washout is initiated at the recommended temperature of 20 degrees Celsius but in cases where ventilation was poor or inadequate during prior cardiopulmonary support. Adequate oxygenation during blood substitution also has the advantage that the patient starts the period of ultra-profound circulatory arrest with adequate metabolic support.

The ATP circuit has a (luer lock) sampling port (on the arterial or venous side or both) and also permits the administration of additional drugs into the reservoir. The sampling ports can be used to withdraw samples for on-site or off-site analysis (to prevent introducing air taking them from the venous side is recommended). For example, the composition of MHP-2 can be verified, or the effluent can be subjected to a series of assays to look for molecules associated with ischemia / cell injury. The option to administer medications can be used to deliver stabilization medications (injected into the reservoir) that were omitted during stabilization due to time constraints or other logistical reasons. It is also possible to administer drugs that directly assist with cardiopulmonary bypass and washout such as vasoactive drugs.

In theory, it is possible to start blood washout at near-normothermic temperatures provided rapid surgery is performed to cannulate the patient and connect him to the perfusion unit. In such a protocol it would not be advised to wash out the blood immediately as the oxygen carrying capacity of MHP-2 (an aqueous solution) would be inadequate to deliver enough oxygen to the patient and the brain in particular. In such a protocol, the patient’s blood would first be circulated and oxygenated closed circuit while being cooled at the same time. As the patient reaches a temperature of ~ 20 degrees Celsius the circuit is opened to wash out the blood. After this step the washout procedure would proceed as usual. It is important to note that in such a protocol careful attention should be paid to the decreasing oxygen needs of the patient as the temperature of the patient is lowered.The team should also monitor (and adjust) other physiological measures such as pH and (cerebral) pressure. Strict conformity to the aim of keeping the patient viable by contemporary medical criteria would probably mandate this kind of procedure.

One aspect of the ATP that has not been discussed in detail is that this device, in principle, can be used, or slightly modified, for field cryoprotection with a vitrification agent, a topic that will be discussed in more detail in the section about cryoprotective perfusion.

Alcor's ATP was never meant to be the final word on a compact, portable, perfusion unit. Since the mid-2000's a new prototype perfusion unit was developed at Alcor that incorporated a hard shell reservoir, a centrifugal pump, and more advanced pressure monitoring options. To our knowledge, this unit has not been deployed in a remote Alcor cryonics case and the project was abandoned when a change in management coincided with a decision to use services from Suspended Animation for Alcor's non-local cases.

Professional Extracorporeal Perfusion Equipment

While the desire to use professional perfusion equipment and medical professionals (surgeons, in particular) has shaped Alcor's objections and procedures since its incarnation, the degree to which this wish has been implemented has varied throughout the organization's history. In a sense, the development of the ATP could be perceived as a departure from using mainstream perfusion equipment while the involvement of Suspended Animation in Alcor's non-local cases returned Alcor to its older practice of using mainstream perfusion equipment and medical professionals – albeit with less control of Alcor over the conduct of these outsourced cases. At the time of the writing of this manual, practically all of Alcor's national remote stabilization cases are performed by Suspended Animation, which contracts with professional surgeons and perfusionists to perform blood substitution in the field. As much as the objectives of the procedure have remained the same , the equipment used in these cases is more advanced. For example, there has been a renewed interest in using state-of-the-art perfusion equipment such as the modular Stockert perfusion unit pictured in Figure 16-6.

While there is a broad consensus within cryonics about the objectives of blood substitution and the required equipment, a number of protocol and design issues are briefly discussed below to conclude this section.

Figure 16-6. The Stockert perfusion system.

Timing of Blood Washout

Alcor's current human cryopreservation protocol recommends that blood washout is started either at 20 degrees Celsius or when the efficiency of

external cooling starts sharply declining (whichever comes first). The rationale for starting washout at 20 degrees Celsius is that this permits the surgeon to establish vascular access for perfusion without the risk of prolonged the ischemia that could occur if this procedure would be started closer to normal body temperature. In many cases, surgical access can only be established at a funeral home or the local cryonics facility and the patient's temperature will be approximating this target by default as a result of transport time. The protocol to simply externally cool the patient to 20 degrees Celsius has been questioned by some for a number of reasons. In the first place, there have been cases in which external cooling and cardiopulmonary support became increasingly ineffective at temperatures above 20 degrees Celsius. In the second place, some people have argued that it should be possible to conduct surgery fast enough to outpace the negative effects of normothermic circulatory arrest. For example, one could imagine a scenario in which the transport medications are administered rapidly and the duration of surgery is shorter than the maximum time these medications are perceived to effectively mitigate warm ischemia. A rejoinder to this argument is that the duration and of surgery cannot always be predicted in advance and it is more prudent to err on the side of caution and conduct surgery at lower temperatures. The issue of the timing of blood washout is highly relevant to the possibility of using mainstream emergency bypass protocols and procedures in cryonics.

Roller Pumps or Centrifugal Pumps

The default option for remote blood substitution and cryoprotective perfusion in cryonics has been to use roller pumps. Some have argued in favor of a switch to centrifugal pumps. One of the major advantages of the use of centrifugal pumps is that they are unable to pump large amounts of air. Another argument is that a roller pump better mimics the pulsatile flow in normal human physiology. Arguments against centrifugal pumps include a higher learning curve, the need for inline flow rate monitoring, and their unsuitability for perfusing high viscosity solutions.

Hard-Shell or Soft-Shell Reservoirs

While soft-shell venous reservoirs have been the preferred form of reservoir for Alcor's ATP, some advisors have advocated to switch to hard-shell reservoirs. Disadvantages of soft-shell reservoirs that have been put forward include: increased volume adds resistance to the venous return line, capable of only limited volumes, difficult to purge air, incompatibility with Vacuum-Assisted Venous Drainage, and inaccuracies in measuring volume. People who favor the soft-shell reservoirs have made the argument that soft-shell reservoirs collapse upon emptying (and thus limit the amount of air that can be pumped) and that their collapsibility allows for more compact perfusion circuits such as the ATP. Michael Darwin has argued that the conjunction of small volume soft-shell reservoirs and increasing tubing length and filter volume(both in contrast to mainstream perfusion) will prevent the maximum volume of air that can be accidentally pumped from being introduced to the patient.

The Arterial-Venous Recirculation Line

The Alcor ATP includes an arterial-venous recirculation line. The objective of this line is to maintain cool temperatures in the circuit in case perfusion needs to be interrupted. It can also be used to purge air from the circuit in case it is caught prior to entering the patient. Without this A/V loop interruption of perfusion would warm up the perfusate and produce air bubbles. Others consider this line redundant or presenting a risk of leaving it open during perfusion, which could even produce retrograde perfusion.

Differential Between Perfusate and Patient Temperature

In cryonics it is routine to expose a patient's blood to a chilled (~ 0 degrees Celsius) perfusate. Some researchers and perfusionists advocate a maximum temperature gradient of 10 degrees Celsius to avoid the microbubbles that are associated with larger gradients. Whether these microbubbles produce clinically relevant pathologies in patients has not been resolved in the literature and there have been no distinct events in cryonics that prompted to revisit current protocols.

Assisted Venous Vrainage

Poor venous return has been observed in some cryonics cases. One suggestion to deal with such cases is to add some type of assisted venous drainage to increase venous return. There are a variety of ways to accomplish such an objective. The most basic approach is gravity-assisted drainage in which the venous reservoir is placed a couple of feet lower than the cannula. One advantage of the portable ice baths with tall legs (as opposed to those that are constructed to be close to the floor) is that they can facilitate such gravity-assisted drainage. In fact, this kind of gravity-assisted venous drainage has been the default procedure in both blood washout and cryoprotective perfusion cases at Alcor. There are two other, "active," forms of assisted venous drainage: Vacuum-Assisted Venous Drainage (VAVD) which is achieved by applying suction to a closed hard-shelled venous reservoir and Kinetic-Assisted Venous Return (KAVD) which is achieved by incorporating a second pump into the perfusion circuit between the patient and the reservoir. Although these approaches have been used with success in mainstream perfusion their use has generally been discouraged in cryonics because of their greater complexity and risk (such as collapsing a vein or introducing air). In case this approach is used it is recommended that the negative pressure applied to the venous line should not be allowed to exceed approximately 40mm Hg.

The topic of assisted venous drainage is also relevant to cryoprotective perfusion, and whole body cryopreservation in particular. Unlike isolated head perfusion, in which the whole stump of the patient's head is available for drainage, good venous return in whole body cases can be challenging and additional strategies may need to be employed to achieve adequate venous drainage.

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