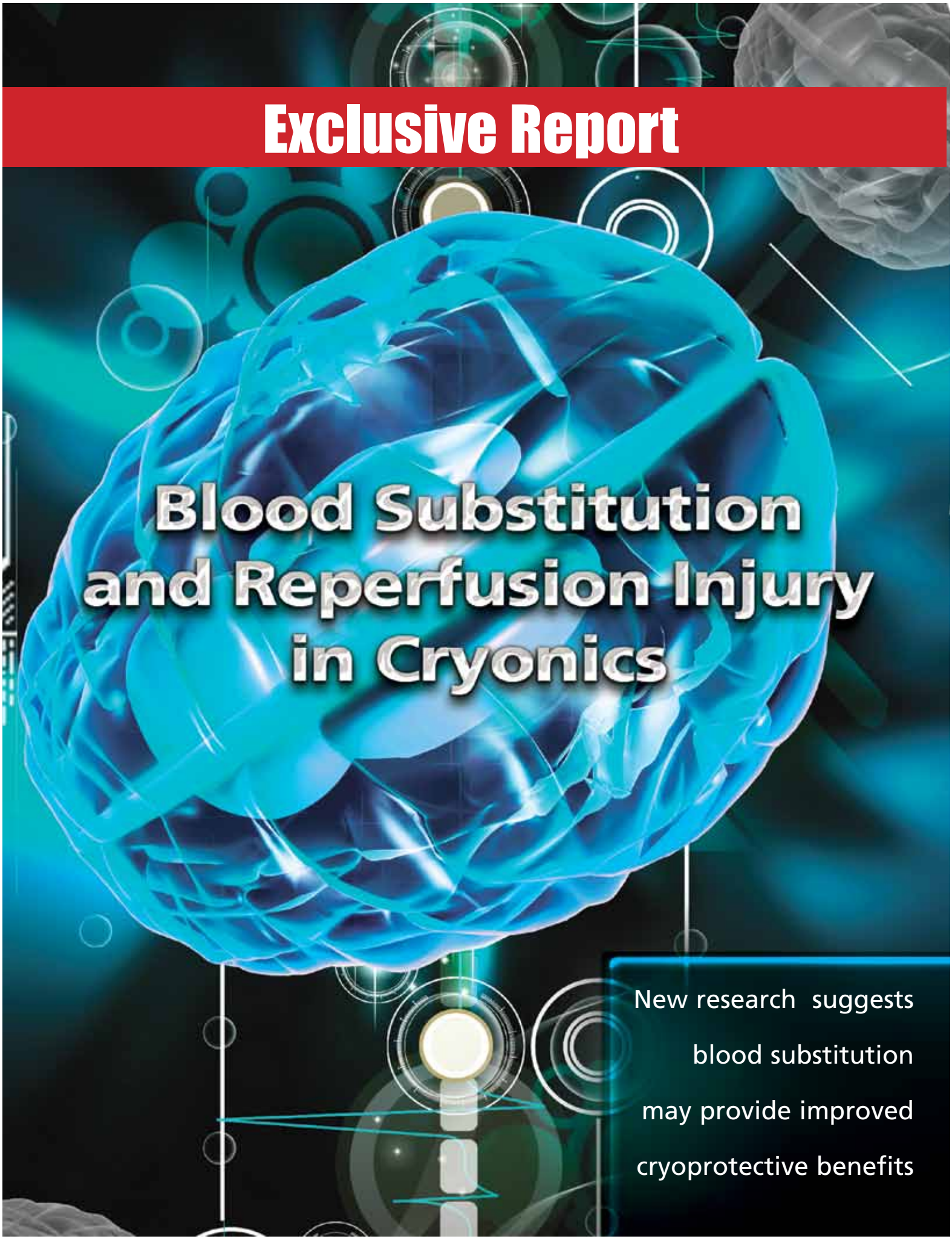
Exclusive Report
Blood Substitution and Reperfusion Injury in Cryonics
New research suggests blood substitution may provide improved cryoprotective benefits

Introduction

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Under ideal circumstances human cryopreservation procedures can be started and completed at the same location (such as a hospital). In reality, patients often need to be stabilized before long-distance transport to the cryonics facility for cryoprotective perfusion and long term care at low temperatures. Because a patient cannot be cooled below the freezing point of water before introduction of a cryoprotectant, the time between initial stabilization after pronouncement of death and start of cryopreservation procedures at a cryonics facility is a time of prolonged cold ischemia. Concerns about (ongoing) blood coagulation, cold agglutination, and recognition of the clinical benefits obtained with organ preservation solutions in conventional organ preservation has prompted some cryonics organizations to provide remote blood washout and replacement with a whole body organ preservation solution prior to transport.

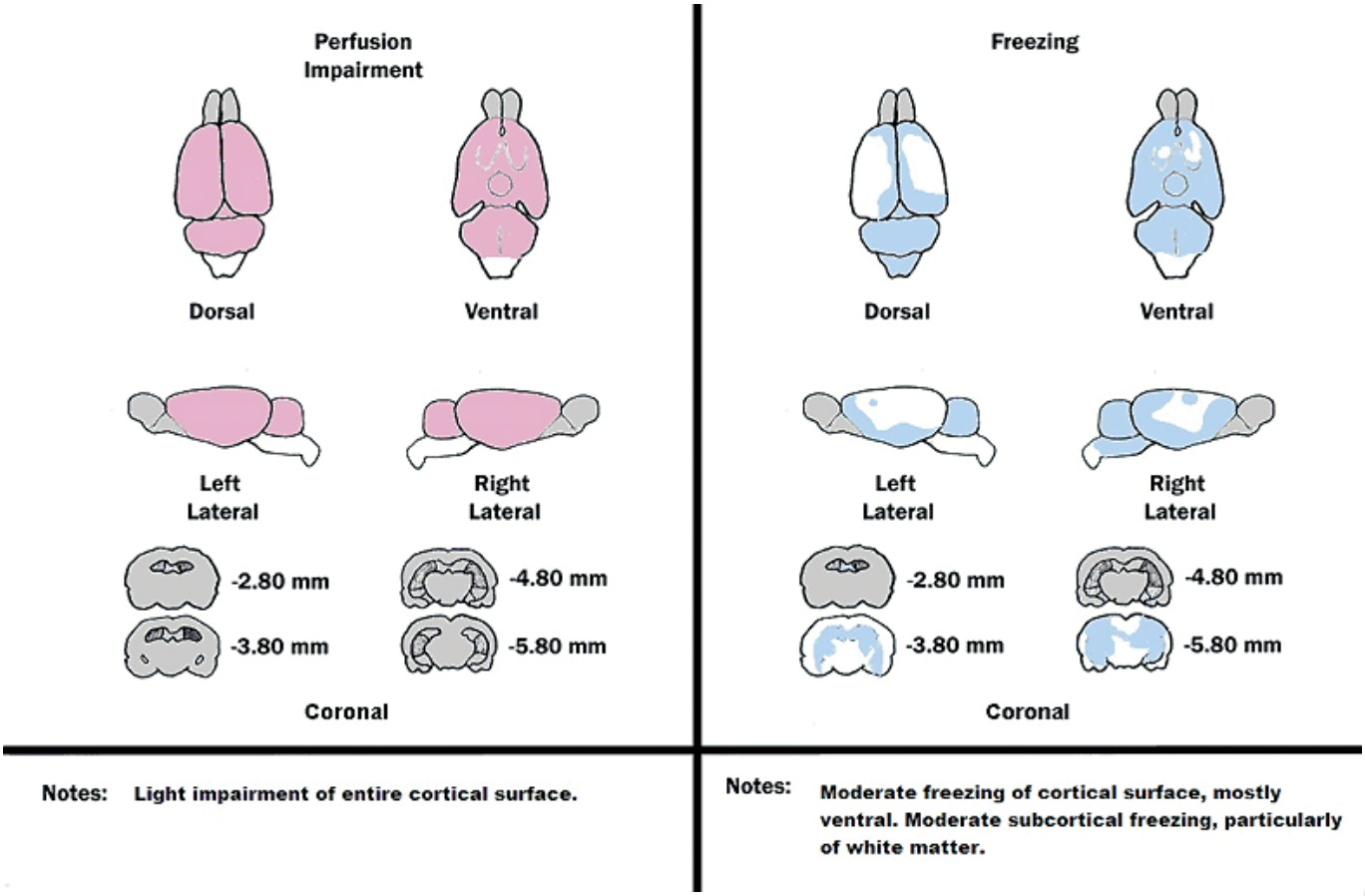
Remote blood substitution in cryonics has a number of important (theoretical) advantages. Replacing the blood with an organ preservation solution extends the period that organs can be recovered from static storage in clinical organ preservation. The procedure also permits a faster cooling rate in the field than is possible with external cooling alone. MHP-2, the mannitol-based perfusate that is currently used by the Alcor Life Extension Foundation, was developed in a series of experiments in which dogs were recovered after up to 5 hours of asanguineous ultraprofound hypothermia (< 5-7°C).

Blood Substitution and Cryopreservation

At the Cryonics Institute, Yuri Pichugin has questioned the value of remote blood substitution in cryonics

Brains maps that document perfusion impairment and ice formation after cryoprotective perfusion and cooling to -130 degrees Celsius

30 minutes of warm ischemia prior to 24 hours of cold ischemia

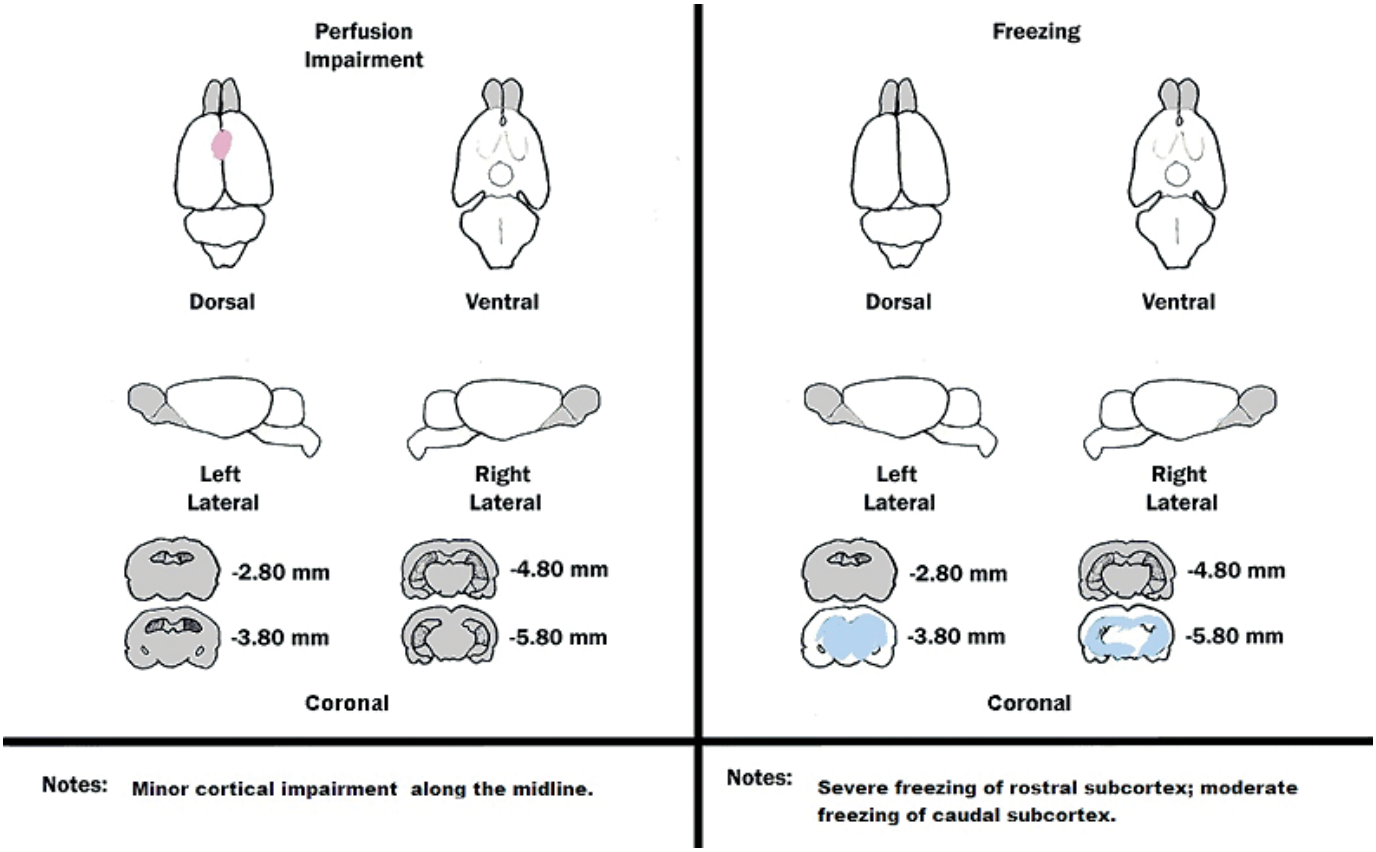


because none of the organ preservation solutions that he tested (including MHP-2 and UW Solution) could maintain viability of hippocampal rat brain slices for periods that represent typical transport times in cryonics. Our own research, however, has been informed by the possibility that remote blood substitution may fall short in terms of preserving viability, but could still confer benefits in terms of improving cryoprotective perfusion after transport.

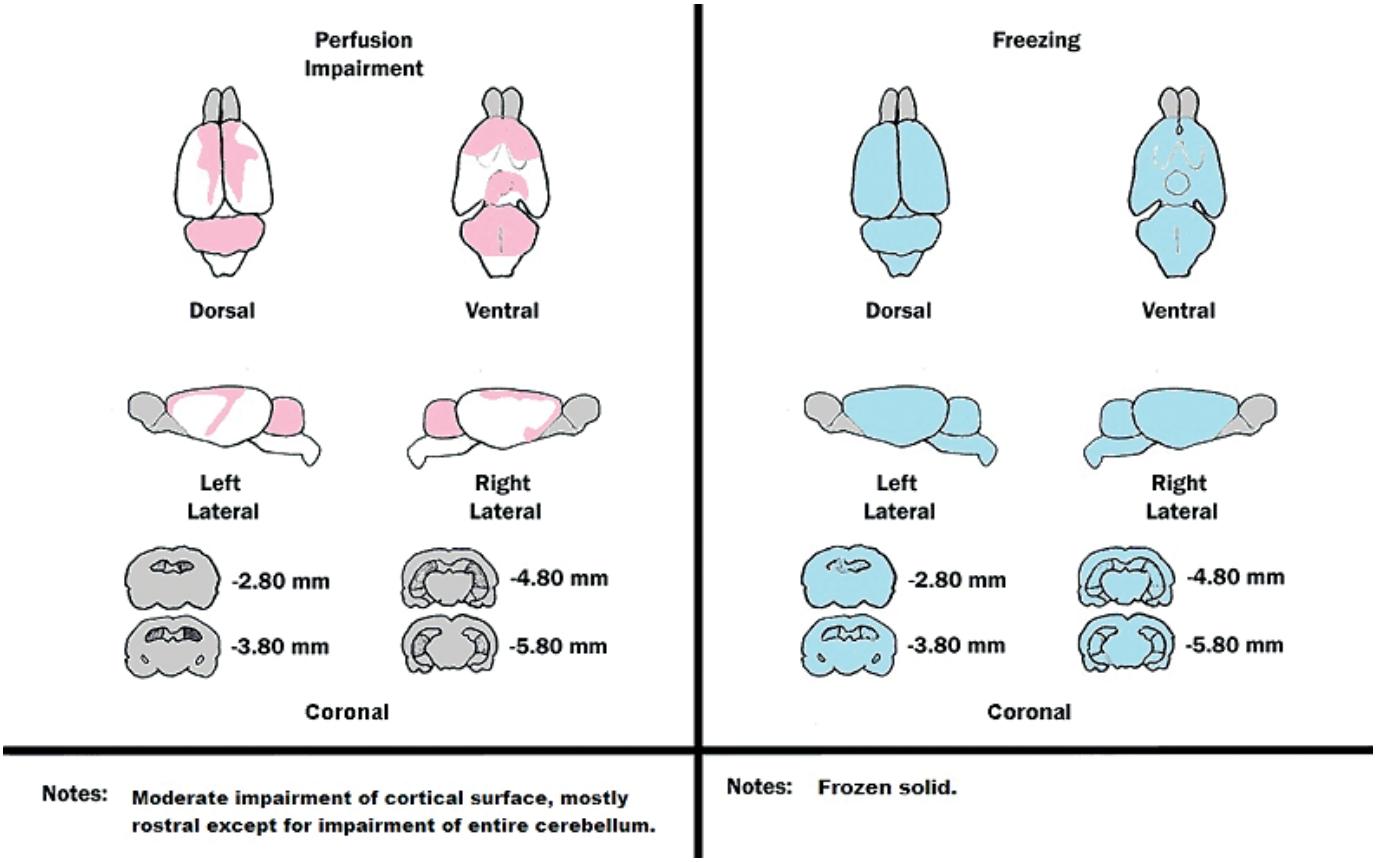
We have compared controls (i.e., no blood substitution) against the following washout solutions: mRPS-2, RPS-2 and MHP-2; and observed that blood substitution does confer significant benefits in terms of improving cryoprotective perfusion and reducing ice formation in cryopreserved neural tissue (rodent). In particular, MHP-2 outperformed the other solutions and has allowed us to conduct cryoprotective perfusion after 48 hours of cold bloodless ischemia with no ice formation in the brain after cooling below the glass transition temperature of the vitrification solution. Even at 72 hours, ice formation is relatively minor compared to 72 hours of cold ischemia in which the blood is left in the brain, which produces severe perfusion impairment and severe ice formation. These experiments vindicate the practice of remote blood substitution in cryonics, but also emphasize that the composition of the organ preservation solution matters a great deal.

None of the organ preservation solutions we have tested (including more advanced recent formulations from colleagues) mitigate the severe whole body vasogenic edema that is observed during cryopreservation after prolonged periods of cold ischemia. This phenomenon has been observed during both VM-1 (Cryonics Institute) and M22 (Alcor) cryoprotectant perfusion. We have designed a number of experiments to improve upon the formulation of MHP-2 but none of these variants has been successful so far in decreasing edema and, in fact, often fared worse than MHP-2 in reducing ice formation after bloodless cold ischemia.

30 minutes of warm ischemia prior to 24 hours of cold bloodless ischemia (washout)



60 minutes of warm ischemia prior to 24 hours
of cold bloodless ischemia (washout)



Delayed Blood Substitution after Warm Ischemia

While our experiments provide further support for the practice of remote blood washout in cryonics, it should be recognized that these experiments were conducted in healthy laboratory rats that did not suffer noticeable warm ischemic injury prior to the start of blood substitution. In cryonics, however, warm ischemic delays are a common phenomenon. This raises the question of whether blood substitution is still effective in cases where there are extensive delays between pronouncement of legal death and the start of blood washout. A related question is whether there is a point where blood substitution does more harm than good. To answer these questions, the Cryonics Institute and LongeCity have supported a series of experiments to get a better understanding of these issues. former CI President Ben Best proposed the initial experiments and collaborated on their design.

In our pilot experiments we investigated the effects of delayed washout, with cortical perfusion impairment and ice formation after cooling to -130° Celsius as endpoints. As the duration of washout delay (i.e., warm ischemia) increased, the degree of perfusion impairment and ice formation after blood substitution with MHP-2 followed by 24 hours of cold ischemia increased as well. After 60 minutes and 90 minutes of warm ischemia there was extensive perfusion impairment and complete freezing of the brain. These results suggest that the effects of warm ischemia cannot be reversed by subsequent blood substitution with an organ preservation solution and that the benefits of blood washout prior to cold ischemia and transport can only be obtained if aggressive external cooling is started promptly after pronouncement of legal death.

We followed these initial experiments with a series of 12 experiments in which we focused on the possibility that

reperfusion injury produced during delayed washout could produce worse outcomes when compared to a protocol without blood washout. To that end, warm ischemic delays of 10, 20, 30, 40, and 50 minutes were followed by 24 hours of cold ischemia or 24 hours of cold bloodless ischemia.

A pair-wise comparison of the experiments revealed that the rats in which the blood was replaced with MHP-2 after warm ischemia and prior to 24 hours of cold ischemia had less ice formation than the rats in which no blood washout was performed. In both groups the degree of ice formation increased as the warm ischemic period increased, which indicates that blood washout cannot reverse the adverse effects of normothermic circulatory arrest but can still confer benefits during cold bloodless storage prior to cryoprotective perfusion. We observed no "tipping point" at which leaving the blood in the animal produces better outcomes than performing a washout.

There are a number of potential explanations for this:

- Decreased periods at warmer temperatures in the washout experiments delayed energy depletion and improved outcome (i.e., the 'cooling' explanation).
- Reperfusion injury is not present, or not present in sufficient magnitude to offset the benefits of blood substitution (i.e., the 'no reperfusion injury' explanation).
- Blood substitution confers independent benefits, aside from rapid cooling, that improve outcome during prolonged cold storage (i.e., the 'hypothermic blood substitution' explanation).

Concerns about reperfusion injury following blood substitution in patients with long periods of warm ischemia are reasonable in light of the published literature on cerebral ischemia and reperfusion injury. The small number of experiments we were able to conduct does not provide the statistical power to completely resolve these issues, but should be useful in the design of further experiments to establish indications and contra-indications for remote blood substitution in cryonics. We observed improved results in the washout protocols after up to 50 minutes of warm ischemia. In experiments where the warm ischemic period was extended to 60 minutes or longer, complete freezing of the brain was observed in both protocols, which indicates that there is a point in human cryopreservation where blood washout cannot confer any benefits, even if it does not introduce additional damage.

Future Research Directions

What distinguishes the use of cardiopulmonary bypass in cryonics from its use in conventional medicine is that the patient is usually not oxygenated during blood washout. This omission of oxygenation during perfusion could be hypothesized to actually prevent the kind of re-perfusion injury that we expected to occur during delayed washout. This hypothesis can be tested by not only comparing washout and no-washout protocols after various periods of warm ischemia but by further distinguishing between washout with and washout without oxygenation.

Another phenomenon that has not been investigated in these experiments is the presence of hypo-perfusion. In cryonics a typical patient undergoes a prolonged agonal period prior to succumbing to disease. Low cerebral perfusion pressures should be distinguished from anoxia and the effect of these conditions on subsequent stabilization procedures such as blood washout remains unknown.

Another concession that was made in our studies was to omit the administration of stabilization medications prior to the start of washout procedures. It is possible that the administration of such drugs would prolong the period of warm ischemia after which remote blood substitution is no longer beneficial.