EXHIBIT 9

Brief Annual Report for Critical Care Research for

2005

Notes Prepared For Saul Kent and Bill Faloon
by Steve Harris, Sandra Russell, Joan O'Farrell.

PROGRESS IN CALENDER YEAR 2005

Critical Care Research had a productive year in 2005.

Chief Projects.

[1] Work on Optimal Liquid Lavage Cooling Strategy During Actual Resuscitation (Non Heart-Beating Dog Model), in Conjunction with Cardiopulmonary Resuscitation Devices

[2] Pilot Project To Serve as Center for Canine Cardiac Transplant, for Testing of Donor Heart Preservation Solutions (In Collaboration with 21st Century Medicine, Inc.)

[3] Hypothermic Asanguinous Perfusion Studies. Restart of Early 1990's Era Project Demonstrating Resuscitation of Dogs After Whole Body Extreme Hypothermia (4 C) for 4 hours, Using Blood Replacement (Organ Preservation) Solutions (In Conjunction with 21st Century Medicine, Inc. Organ Flush and Storage Preservation Project)

[4] Continued Dog Breeding and Experimental Dog Vivarium Management.

[5] Complete Overhaul of Surgical Suite and Associated Equipment, in Support of Cardiac Transplant and Hypothermic Asanguinous Perfusion Studies.

SUMMARY PROGRESS

PROJECT 1: Work on Optimal Liquid Lavage Cooling Strategy During Actual Resuscitation (Non Heart-Beating Dog Model), in Conjunction with Cardiopulmonary Resuscitation Devices

We have purchased a Zoll AutoPulse CPR system ($15,000). This system consists of a board on which a patient is placed, with a plastic strap and pad which is adjusted about

the chest. When the digitally-controlled battery-powered electronics in the board are triggered, a computer senses tension in the chest straps, and uses an electric motor to initiate CPR, by alternately winding up (tensioning) the straps, then releasing them. Blood pressures of about 60 mmHg (Tor) are achievable by this method in humans in cardiac arrest. The AutoPulse is portable and in use by paramedic teams in the U.S. and many countries. See http://www.zoll.com/product.aspx?id=84. The AutoPulse competes with manual methods of CPR. The device was pre-clinically tested in swine, and it was unknown if it would work at all in dogs.

In our series of tests of the device (the first in dogs) we found that the device could be made to provide blood pressures up to 45 Tor in animals in cardiac arrest, and worked best if the dog was placed on its side. In this series of experiments dogs were used which had been placed into cardiac arrest after anesthesia, with an A.C. heart-fibrillating shock. The AutoPulse was then used to maintain blood pressure by CPR, and cooling was attempted with cold perfluorocarbon lung lavage. The animals did not survive these studies (no actual resuscitation was attempted).

Early findings in this series include:

1) Perfluorocarbon lung lavage modestly increases blood pressure achievable by the AutoPulse. The mechanism appears to be decreased chest-compliance, as caused by filling the lungs with fluid. Tests of other CPR devices with inspiratory ITD (Impedance Threshhold Devices) have suggested that the lungs can serve to counteract CRP if air is allowed to flow into and out of the chest, rather than blood. http://www.circ.ahajournals.org/cgi/content/abstract/108/18/2201 We have found that filling the lungs with oxygenated fluid acts modestly like an ITD device, and indeed we found that clamping the ET tube during chest compressions did not add to the liquid effect.

2) We tested the maximal rate of cooling in a dog in cardiac arrest, by means of lung lavage and CPR as delivered by the AutoPulse device. We found that lack of a normal cardiac output did greatly limit the rate of brain cooling in cold lung lavage, although brain-cooling rate was still much faster with CPR and lung lavage, than it was with only simple ice packing of the head of an animal in cardiac arrest.

In previous experiments we have found that lung lavage applied in a non-injurious manner consistently delivers at least 0.1 C/min cooling rate early in cooling (i.e, with at least 30 degrees C temperature difference between cooling fluid and brain temperature), and that the maximal cooling rate may be as high as 0.5 C/min. By contrast, with AutoPulse assisted CPR in the dog, lung lavage was only capable of delivering venous cooling of about 0.1 C/min, and that the brain cooled at half this rate, or less. A more positive outcome was that arterial blood oxygenation was maintained by a combination of lavage cooling and 100% oxygen ventilation. As in our previous studies, we found that gas ventilation was sufficient to maintain oxygenation, and no separate liquid oxygenator was required.

An overall conclusion is that the dog's chest is probably not a sufficiently good model for CPR in the human, and that pigs or human cadavers must ultimately be used to test the role of the AutoPulse in fluorocarbon-lung cooling (cooling during CPR, or for cryonics).

PROJECT 2: Pilot Project To Serve as Center for Canine Cardiac Transplant, for Testing of Donor Heart Preservation Solutions (In Collaboration with 21st Century Medicine, Inc.)

The objective of this pilot study is to test whether it is feasible, in collaboration with **21st Century Medicine (21CM)**, to perform survival dog heart allotransplantation surgeries at **Critical Care Research (CCR)** and to establish the 1-week survival of heart transplant recipients which have received a donor heart with either minimum storage time or overnight storage.

If this pilot study shows that survival dog heart transplantation can be done successfully at **CCR**, a survival dog heart transplant model will be set up at **CCR** to test a storage method recently developed at University of Rochester that can preserve the viability of the donor heart for extended period (24 hours or longer).

The present clinically acceptable duration of donor heart storage is 4-6 hours, which severely curtails the availability of donor hearts. With the improved storage method recently tested at **University of Rochester** (**UR**) and licensed to 21CM, the storage time may be extended to 24 hours or more, without compromising the well-being of the donor heart. This has (up to the present time) been proven only in dogs temporarily given donor hearts for a few hours, as the University of Rochester was never able to control bleeding and other factors in the post operative period well enough to allow transplanted dogs to be weaned from the ventilator and survive.

If the increased storage time demonstrated by the UR solution is successfully translated into an animal survival model, it can be tested in humans. If successful in humans, a 24-hour storage time will translate into a significant increase in the availability of donor hearts, at least half of which are now discarded because, even though usable in theory, the organs exceed the time limit available before they can be sent to a donor.

The canine cardiac transplantation model has been well established in the literature for the evaluation of heart preservation. Selecting dogs for testing of UR heart preservation solution has the potential to shorten the learning curve in development, since many direct human techniques in bypass and cardiac surgery can be used directly without modification, in the dog model. Mongrels (mixed breed domestic dogs) such as are bred at Critical Care Research, Inc. are of sufficient size to allow reasonable surgical access and manipulation.

[Note on autotransplantion (taking out a heart and putting it back into the same animal it came from). Although autotransplantation can reduce the number of animals to be used, autotransplant in practice is more difficult to perform due to anatomical limitations. In autotransplant, the heart has to be excised, trimmed, and sewed back perfectly. There is

practically no room for remedial measures and the risk of surgical complications greatly increased. In addition, if the newly developed preservation method is to be tested over long periods of time, the autotransplantation model cannot be used, because this would require maintaining the recipient animal on cardica bypass for extended times such as 24 hours. Therefore, allotransplantation is needed, using pairs of animals].

Five pairs of allotransplants (10 dogs) should provide enough evidence to support whether Critical Care Research, in collaboration with 21st Century Medicine, has the capability to start a survival canine cardiac transplant program. This number of animals use may be reduced if our success rate is over 50% with the first 3 experiments (6 dogs) and the IACUC agrees that no more transplants is necessary for this pilot study.

ONE TRANSPLANT has been attempted at Critical Care Research, Inc. After a team from CCR visited the University of Rochester and viewed an attempted (but failed) dog transplant there, materials and a team for the transplant were assembled at CCR. A number of dogs were put on femoral cardiac bypass and cooled to 20 C, with rewarming and survival of all. After this necessary experience, a team was assembled led by a human pediatric cardiac surgeon (Nahidh Hasaniya, MD., assistant Professor of Surgery at Loma Linda University, Division of Cardiothoracic Surgery, School of Medicine), and Ross Lirtzman, DVM, a veterinary thoracic surgeon. Dr. Hasaniya had never done a transplant in a dog (but had performed it in swine and goats) and Dr. Lirzman had done chest surgery in many dogs but never a heart transplant. However, together they brought the necessary experience to attempt the procedure.

The outcome was not successful, but came close enough to success, to continue the program. A heart was implanted which had been preserved overnight in UR solution, far longer than cardiac surgeons ever seen successful implantations. Yet when implanted, this heart functioned and gave normal blood pressure, allowing the animal to be weaned from bypass. Unfortunately, shortly after closure of the chest, the animal died for reasons which were not fully apparent on autopsy. Another attempt has been scheduled.

PROJECT 3: Hypothermic Asanguinous Perfusion Studies. Restart of Early 1990's Era Project, Demonstrating Resuscitation of Dogs After Whole Body Extreme Hypothermia (4 degrees C) for 4 hours, Using Blood Replacement (Organ Preservation) Solutions (In Conjunction with 21st Century Medicine, Inc. Organ Flush and Storage Preservation Project)

This series of ultraprofound hypothermia (below 15 C-- in the region of cardiac arrest) experiments were originally termed ***hypothermic asanguinous perfusion*** experiments by Darwin and Leaf, who performed them from 1984 until the series was terminated by Leaf's death in 1991. They were continued by Darwin, Harris, and Russell at **21st Century Medicine** until 1995, when they were again discontinued after the laboratory moved to its present location in Rancho Cucamonga, and cardiac arrest and post-resuscitation (mild) hypothermia experiments begun (including the liquid ventilation lung-cooling series of experiments).

In the ultraprofound hypothermia experiments, a dog is anesthetized, cooled on cardiopulmonary bypass to 10 C (far below the range at which cardiac activity ceases), then has its entire blood volume removed and replaced with an iso-osmotic electrolyte solution containing a colloid, such as 6% hetastarch. Perfusion is continued in the temperature region from 4 C to 7 C, at pressures of about 30 mmHg for 4 hours. After this time the dog is warmed, the solution is replaced again by blood, rewarmed further, and the heart restarted by defibrillation, as soon as electrolyte re-balance has been achieved. The procedure has been successful for as long as 5 hours, but this has been the limit in the past, and is set by pulmonary edema and brain damage if perfusion at low temperatures is continued longer.

Rationale: Currently, clinical circulatory arrest times for human neurosurgery utilizing hemodilution and profound hypothermia are limited to a maximum of 1-hour At 15 C. Even with this comparatively brief period of cold ischemia, serious complications in the form of coagulopathies and both reversible and irreversible neurological deficits frequently occur. This relatively brief period of circulatory arrest does not allow for the execution of complex neurosurgical procedures such as tumor resection necessitating interruption of blood flow, or vascular reconstruction secondary to aneurysm resection or repair of aneurysm rupture. The scope and complexity of such procedures might be expanded significantly, if circulatory arrest times could be extended.

Similarly, multi-organ harvesting from a single donor would also be facilitated if an effective and reliable means could be demonstrated to preserve cadaver donors by perfusion or by flush-store techniques for an extended period of time. In particular, harvesting of hearts and lungs, with transplant periods limited presently to six hours at ice temperature (equivalent to about 20 minutes at body temperature), might be considerably extended if the body temperature time of ischemia prior to removal could be decreased. Presently this period, even in the best of circumstances, may be a considerable fraction of 20 minutes, and thus may contribute to the present surgical limit on storage of these organs after conventional cadaver recovery.

Work conducted by this laboratory in the mid 1980's documented the successful recovery of adult dogs from four-hours of asanguineous perfusion at 4 to 5 C following total body washout (TBW) employing an intracellular solution (i.e., potassium concentrations of about 28 mEq) containing mannitol as the impermanent agent and sodium HEPES as the buffer (MHP-1). This work also disclosed the evidence of frequent neurological injury of a time-dependent nature, apparently as a result of cold ischemia. MHP-1 was not completely effective in eliminating cold-ischemic injury to the CNS, as evidenced by the presence of post procedure seizures, visual disturbances, (including temporary blindness), and hind limb weakness. While these side effects resolved over a period of 1-3 weeks, they were and are indicative of CNS (brain and/or spinal cord) injury. In addition, it was observed that as a-sanguinous (blood free) perfusion time was increased from 2 to 4, and then to 5 hours, both the frequency and severity of these neurological side effects increased.

While MHP-1 was effective in providing reasonably good protection during 4 hours of

asanguinous perfusion, no systemic studies have ever been conducted to determine the time limits of its effectiveness or possible ways to improve either the solution, or the TBW protocol as a whole. Now that **21st Century Medicine, Inc**. has proposed to develop organ preservation tools to preserve all organs from a research animal for use in tissue slice pharmaceutical models, as well as a program to use all organs from human cadavers via pre-perfusion of the body with cold solution before organ harvest, Critical Care Research, Inc. has again become involved in the dog model of ultraprofound hypothermia.

Restart of Asanquinous Perfusion Research. In 2005 we revisted the previous protocol, using our newly acquired Sarns 8000 bypass system. We first demonstrated simple bypass cooling of several canines to 20 C (68 F) and rewarming from that temperature. (This amount of cooling did not, and normally does not, cause cardiac arrest in dogs). These animals recovered almost immediately, with no neurological or pulmonary sequelae.

Next, an animal was cooled to 7 C, after complete blood replacement starting at 10 C. Cardiac arrest occurred at 15 C. Blood was replaced with MHP-1 solution according to the exact formula of the Leaf/Darwin studies. After 4 hours of cardiopulmonary bypass at a pressure of 30 mmHg, the animal was rewarmed and blood was replaced (including donor blood). This animal was easily defibrillated into normal sinus rhythm, but experienced pulmonary edema at the end of bypass, which responded to transfusion with large amounts of fresh frozen canine plasma (available from canine veterinary blood banks). For a week following resuscitation, the dog also exhibited the classic type of reversible neurological injury seen in the earlier series of ultraprofound hypothermic asanguinous perfusions: cortical blindness and dyscoordination. However, these completely resolved in about a week, leaving a fully intact dog which survived long-term, showing that the earlier performance of this solution and this system was still reproducible. Several aspects of our newer system proved easier than the previous series of experiments, which had used renal dialysis to lower potassium concentrations after perfusion with high-potassium type intracellular solutions such as MHP-1. (For this experiment, we were able to lower potassium into a range permitting defibrillation using a simple technique of isovolemic blood/plasma ultrafiltration against normal saline, called Zero Balance Ultrafiltration or "Z-BUF").

Next, we checked the performance of a newer generation of intracellular-type organ preservation solutions, in this whole-body model. The first of these was **Tran Send**, a solution designed at 21st Century Medicine, Inc. to preserve kidneys in cold storage, using the rabbit model. For the experiment in a whole dog we modified **Tran Send** with colloid to be compatible with a whole-animal system. Although **Tran Send** had never been designed to be compatible with any organ other than the kidney, we had some hope that it might not cause undue injury to other organs, since it designed to be a high potassium "intracellular type" organ preservation solution, in many ways similar to MHP-1.

The result was unexpectedly good. With **Tran Send** we saw spontaneous restarting of

cardiac function, once potassium levels had been lowered below 10 meq/L. In addition, our period of dyscoordination lasted less than a day, with only several days of cortical-type visual problems. Again, the dog survived without any deficit or damage, and fully normal function of lungs, brain, liver, and kidneys, according to lab testing. This test along suggests the possibility of "multi-organ" storage solutions which can be used to perfuse brain-dead cadavers, allowing harvesting of hearts, lungs, and many other organs without specially organ-specific harvest-perfusion techniques.

Finally, in a demonstration experiment in this series designed to test the hypothesis that many "single-organ" preservation solutions can be used in a whole animal model for many organs, we repeated the 4-hour hypothermic perfusion using University of Rochester Heart Preservation (UR) solution, again modified with colloid. This solution was designed to preserve hearts only, and contains not only no calcium, but also EDTA as a calcium-chelator. It also contains 2,3 butanedione-monoxime, a powerful muscle paralytic which is intended to block rigor-like muscle-hardening in ischemic hearts. It is also an extracellular type solution with a potassium in the range of 5 mEq. It has never been used to attempt to preserve any organ but the heart. We do not believe it had ever been given to a living animal.

Nevertheless, when this solution was used for a whole animal to replace the blood volume in a hypothermia experiment at CCR, again the animal ultimately survived the procedure, although large amounts of calcium had to be given in resuscitation, and the animal suffered difficulty in defibrillation and exhibited abnormalities in jaw-muscle tone and also a histaminic reaction which may have been an unrelated allergy. However, this experiment demonstrates that probably a very broad range of electrolyte solutions, so long as they contain colloid and appropriate protein, can be used to preserve all organs (including the brain, so long as minimal oxygenated perfusion at 30 to 40 mmHg is maintained), for at least 4 hours. In our next year, therefore, we hope to combine existing organ preservation solution types, to identify solutions which may be used for:

1) Preservation of whole animals with no blood pressure for at least 3 hours at 0 to 2 C, without permanent brain damage (and of course no permanent damage of other less ischemia-tolerant organs).

2) Preservation of whole animals with 40 mmHg asanguinous perfusion for at least 6 hours, and preferably 8 hours, thus firmly establishing that the present 4-5 hour limit is due to unacceptably low perfusion pressures which are the present result of high central venous pressures and high viscosity with the current solutions. In the process of testing various organ preservation solutions in the whole animal model, we have also identified methods of whole body perfusion which minimize central venous perfusion pressures at low temperatures. This in turn allows higher perfusion pressures (which allow preservation of the brain) without induction of limiting pulmonary edema.

Note on neurological injury in dogs. A previous series of resuscitation experiments from complete cardiac arrest at 37.5 C in our laboratory, demonstrated survival of animals from up to 16 minutes of no blood circulation complete circulatory arrest.

However, these animals were initially apparently severely neurologically damaged, requiring extended nursing care (these animals were unable to drink or hold their heads up for a minimum of a week). Yet all animals which survived, did so with NO permanent neurological injury whatsoever. These animals all regained the ability to run, and ability to be trained in normal tasks, such as finding food. Similar results were obtain in our late series of ultraprofound hypothermia dogs, in which all three animals required extended nursing care (a minimum of 2 days of IV fluids before being able to drink, and inability to stand for up to a week) and yet all survived with no neurological sequelae. We believe that a great many animals labeled as "profoundly neurologically damaged" in resuscitation studies actually are minimally damaged, and have the prognosis for full recovery, if given adequate care in the recovery phase. However, ability and will to perform critical or intensive-care style nursing for animals is limited in the usual research setting, and this artificially limits animal models in many ways which are completely avoidable. We intend to argue this point in future publications.

PROJECT 4: Dog Vivarium and Model Development, Breeding Program, and Community Outreach.

Our dependence on outside "class B" dog dealers as a source of experimental animals was ended in 2001, as we began a program of complete in-house breeding of dogs, which were to be raised from puppies through adulthood at our facilities. At present, we maintain about 60 dogs in all stages of development, in complete indoor controlled environment kennel facilities overseen by a veterinarian and regularly inspected by the USDA. Our breeding program has generally been successful, and we have had good relations with the local veterinarians. This is partly as a result of using our colony as a source of donated dog blood to save injured dogs in a local vet practice. This does not harm our colony, and makes for better public relations. We benefit also from having our inspecting vet take time on his inspection days to do cat and kitten spay and neutering for a local cat adoption group. This benefits both the vet and the local cat-advocacy group, which knows that we don't use cats in any of our experimental programs.

PROJECT 5: Complete Overhaul of Surgical Suite and Associated Equipment, in Support of Cardiac Transplant and Hypothermic Asanguinous Perfusion Studies.

We have recently completely refurbished and remodeled our two operating suites, the O.R. stockroom, and the animal I.C.U. This work included complete repainting, ceiling tile replacement, and floor work.

In addition, we have been able to update our cardiovascular bypass capability with a donated Sarns 8000 heart-lung machine which replaces our Sarns 7000 machine. In addition, a new Sarns heat exchanger was given along with the machine, which has the capability of very rapid heating and cooling for inline cooling of blood and blood replacement circulatory solution in whole body hypothermia experiments. These systems (which cost $80,000 new and are still worth about $25,000) were donated by Riverside Community Hospital in response to our program of cardiac transplantation heart preservation solution development, as detailed above.

Other recently acquired equipment includes:

* CDI 100 inline blood gas monitoring to allow for continuous blood gas monitoring while on cardiac bypass

* Narkomed 2B anesthesia workstation and ventilator system to replace Ohio anes. system and ventilator

* Respironics NICO 2 non-invasive cardiac output monitor to augment our present COS2MO system. This will be used to monitor dog cardiac output after transplant in our planned series.

* Haemonetics **Cell Saver 3** machine with capability to recover blood and wash red cells used in both cardiac transplantion and whole body washout experiments

* Stainless steel glass fronted cabinets to replace open shelving

* New X-ray processor to replace broken processor

*New set of O.R. lights rewired to replace broken/aged wiring

* Set of complete surgical instruments and disposables acquired specifically for heart transplant operations

* Heart-lung bypass disposables (ie, many donated sets of disposable hollow fiber oxygenators and heat exchangers for washout studies and heart tranplant studies)

* New Zoll Autopulse automated CPR machine acquired to CPR studies to evaluate CPR and rapid cooling with liquid ventilation

SUMMARY. The dog remains a particularly valuable animal for the testing of resuscitation strategies, with the caveat that the dog's very deep and prow-shaped chest make it a poor model even for band-type constriction CPR devices. However, we continue to be able to easily perform femoral cannulation for temperature and blood gas monitoring in dogs, and to perform cardiac bypass in dogs. These procedures make the dog an ideal subject for studies in which cardiac bypass is employed: profound cooling studies, resuscitation studies, and (recently) cardiac preservation and transplant studies. These studies promise to provide early payoff in organ preservation solutions for the heart and lungs, as well as techniques for profound whole-body cooling which come closest to providing temporary suspended animation for the brain.

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Prepared for Critical Care Research

Brief Annual Report for Critical Care Research for

2007

Notes Prepared For Saul Kent and Bill Faloon
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PROGRESS IN CALENDER YEAR 2007

Critical Care Research made major progress on several fronts in 2007.

Chief Projects.

[1] Work on Optimal Liquid Lavage Cooling Strategy, with extremely rapid cooling rates achieved.

[2] Work on the first Truly Portable High Capacity Lung Lavage Device

[3] Pilot Project To Serve as Center for Canine Cardiac Transplant, for Testing of Donor Heart Preservation Solutions (In Collaboration with 21st Century Medicine, Inc.)

[4] Continued Dog Breeding and Experimental Dog Vivarium Management.

SUMMARY PROGRESS

PROJECT 1: Work on Optimal Liquid Lavage Cooling Strategy, with a strategy developed for extremely rapid cooling rates.

In previous experiments we have found that cold perfourocarbon lung lavage in dogs applied in a non-injurious manner consistently delivers at least 0.1 C/min cooling rate to the brain early in cooling (i.e, with at least 30 degrees °C temperature difference between cooling fluid and brain temperature), and that the maximal cooling rate may be as high as 0.5 °C/min (see Harris, S.B. Darwin, M.G., Russell, S.R., O'Farrell, Fletcher, Mike, and Wowk, Brian, Rapid (0.5°C/min) minimally invasive induction of hypothermia using cold perfluorochemical lung lavage in dogs. *RESUSCITATION* 50: 189-204, 2001. PMID: 11719148). This cooling rate was achieved with a type of lung lavage which was a-synchronous with breathing, due to the requirement for gas ventilation at least once every 15 seconds, and the difficulty in complete delivering and removing a large bolus of

cold perflurocarbon in this time. One the other hand, small boluses of fluorocarbon were found to transfer heat less efficiently in our model. For example, the maximal cooling rate achieved in this paper was achieved at a perfluorcarbon lavage rate of 31 mL/kg/min, but this required a lavage time of 37 seconds. The lavage volume for this maximum was 20 mL/kg, but when this was dropped to 9 mL/kg (allowing a lavage time of 16 seconds) the heat transfer efficiency dropped from 60% to 40%, and consequently cooling rates dropped by 30%, even though the lavage rate had increased slightly to 36 mL/kg/min. At the time, we suspected that we had reached a limit, which was due to diminishing heat transfer efficiency, and mechanical efficiency of removal, with small lavage volumes.

In subsequent work, however (detailed in our previous reports) we found that the drop in heat transfer efficiency was avoidable if perflourocarbon infusion was synchronized with oxygen gas insufflation (best provided by hand bag-valve unit), so that cold perfluorocarbon could always be provided into lungs being expanded by insufflation of pure oxygen gas. We were able to show heat transfer efficiencies as high as 50%, even with lavage volumes as low as 3 mL/kg, with this technique. However, limitations in our equipment (primarily in suction efficiency) still prevented us from conducting lavage faster than 20 mL/kg/min at these small lavage volumes. From theoretical considerations, we understood that we would need total lavage rates of about 60 mL/kg/min to achieve brain cooling rates in the neighborhood of > 1 C/min.

Challenge of Becker, et al. In 2007, Lance B. Becker and Joshua W. Lampe published a paper in Expert Rev. Med. Devices 4(4), 441-446 (2007) in which they challenged experimenters to design a cooling system which would lower brain temperature by 4°C in the first 5 minutes of applications. They wrote:

"The induction of mild hypothermia, lowering body temperature by 4°C, is gaining acceptance as an acute therapy for the treatment of hypoxia and ischemia following cardiac arrest and many life-threatening injuries. When hypothermia is used following ischemia (as opposed to before ischemia), it needs to be performed rapidly for the greatest benefit, preferably within 5 min. A simple model reveals that this poses a significant bioengineering challenge as the rate of heat transfer is severely limited, owing to a relatively confined fundamental parameter space. Current methods of cooling include external cooling devices, such as cooling blankets or ice bags, which are simple to use, relatively inexpensive but slow. Internal cooling has the best ability to cool more rapidly but current devices are more invasive, costly and most are still not able to provide cooling within the rapid 5-min interval."

Lampe and Becker go on to a discussion of theoretical amounts of heat which can be removed by a number of methods, including cardiac bypass, but note that this takes at least 1 hour to implement. They conclude:

"Hypothermia of 3-4°C must be achieved within 5 min for maximum protection when required after a period of ischemia. The human body is well designed to prevent heat loss, creating a significant bioengineering challenge in a relatively confined fundamental parameter space. The minimum temperature and the maximum volume of coolant that

can be introduced into the body are significant engineering obstacles."

In reading Lampe and Becker's article we realized that they are seriously miscalculated the amount of heat which would be need to be removed during rapid cooling, due to their inexperience with the decrease in effective heat capacity of the body, which occurs during very rapid cooling of the blood. In effect, our previous experiments had found that only a 70% "core" mass (which includes the brain, fortunately) is cooled in the first few minutes of cooling, and that if cooling continues past 5 minutes, the extra heat to be extracted in the 6^{th} and 7^{th} minute will make up for the "re-equilibration" which takes place within the animal or human, as the cold core and brain are re-warmed by the periphery, which does not initially cool as fast as they do. Thus, we knew that Lampe and Becker's estimates of needed heat extraction were off by a factor of 2, and also that their estimations of the amount of cold fluorocarbon which could be delivered to lungs was incorrect.

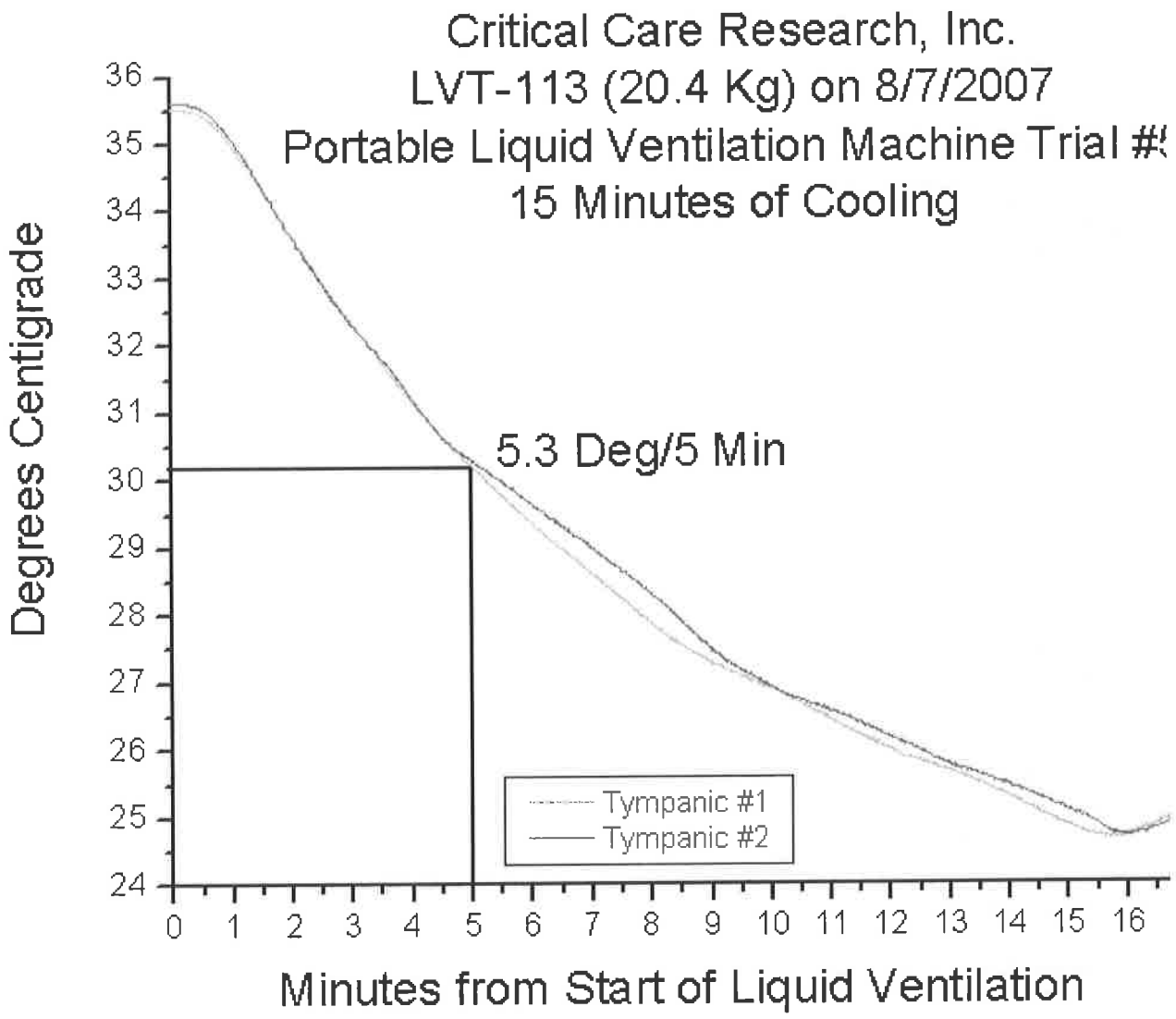
In our last report to the Life Extension Foundation, we detailed problems we had had with the breadboard prototype lung lavage machine designed by SPECK corporation. Although this machine featured fully peristaltic type pumps for both fluorocarbon infusion and suction (these work by massaging the outsides of a tube, as in a heart-lung machine) they were not able to provide the very high suction rates which were required to return 60 ml/kg/min of PFC, which we estimated would be the minimum to achieve Lampe and Becker's goal of -4 °C in 5 minutes (which we knew would require -1 C/min, since there is very little brain cooling in the first minute of blood cooling by any device, due to heat exchange delay).

[2] Work on the first Truly Portable High Capacity Lung Lavage Device

Accordingly, beginning in 2007 we contracted with Gary Battiano and Charles Platt at Suspended Animation, Inc. (also funded by the Life Extension Foundation) to provide a suitable high capacity (high suction) machine, which would be completely portable (able to run from batteries) and also able to cool more than -4 C, should this be required. In designing this machine we made use of the high-capacity commercial "plate-type" heat exchangers described in our last report (capable of nearly 600 watts/degree heat transfer between icewater and cold perfluorcarbon). Battiano and Platt also discarded the bulky peristaltic pumps and replaced them with inexpensive commercial fluid diaphragm pumps, which proved far superior, and able to run on low voltage D.C.

With the superior suction capabilities of this device, combined with its ability to provide a constant supply of perfluorocarbon cooled to 2 °C (from a reservoir containing only 7 liters of fluid) we performed a series of 20 lung-lavage dog experiments in 2007 and early 2008. The first results were encouraging enough to apply for a preliminary patent on the device in October 2007, and a full patent application was filed (Platt, Battiano, Harris) for the device in October, 2008.

A graph from one of the early experiments with the device, after design parameters had been adjusted, illustrates performance:



This graph shows that Lampe and Becker's stated goal (which they thought impossible without a direct application of a phase-change substance to the body) of -5 °C in 5 minutes has been exceeded with the device. In addition, in this experiment, we were able to cool the animal's brain and heart to 25°C (77 °F), in just 16 minutes.

Later experiments with the device show that 6-7 minutes of application of 60 mL/kg/min lung-lavage with ice-cold perfluorcarbon (2 °C) results in at least -4 °C drop in brain temperature in the first 5 minutes, and that application of lavage for more than 6 minutes results in a permanent body core temperature of -4 °C. All of these results are novel, and

should be of great importance to the resuscitation community. A formal report on them is now being prepared for publication, now that the patent application has been submitted.

Animals subjected to only -4°C of cooling (enough to trigger post-resuscitation brain protection, according to many other studies) do particular well in this model. When allowed to survive for the long term, they at first have minimal symptoms (slight airway obstruction) which is not severe enough to keep them from walking and eating. Thereafter they recover completely, after the first week. In animals which we have sacrificed at 48 hours to look at lung pathology, we see minimal or no hemorrhage indicative of pressure-damage. We are able to keep total lung pressures low (< 30 cm H20) in this model, mainly by keeping lavage volumes to below 9 mL/kg (the oxygen gas inspiration volume is about twice as much, or 20-25 mL/kg). Currently we are routinely running 8-9 mL/kg lavages at 8 seconds/lavage (7.5 breaths and lavages/minute), for total rates of 60-68 mL/kg/min.

PROJECT 3: Pilot Project To Serve as Center for Canine Cardiac Transplant, for Testing of Donor Heart Preservation Solutions (In Collaboration with 21st Century Medicine, Inc.)

The objective of this pilot study was to test whether it is feasible, in collaboration with **21st Century Medicine (21CM)**, to perform survival dog heart allotransplantation surgeries at **Critical Care Research (CCR)** and to establish the 1-week survival of heart transplant recipients which have received a donor heart with either minimum storage time or overnight storage.

If this pilot study showed that survival dog heart transplantation could be done successfully at **CCR**, a survival dog heart transplant model would be set up at **CCR** to test a storage method recently developed at University of Rochester that can preserve the viability of the donor heart for extended period (24 hours or longer).

The canine cardiac transplantation model has appeared to be well-established in the literature for the evaluation of heart preservation. However, we later found that all dog heart transplant models had been stopped, and the work was being done on goats. We were unable to locate any experienced dog heart transplant surgeons, so we utilized trained experienced dog thoracic surgeons and a pediatric heart transplant surgeon from Loma Linda medical center.

In our series of 6 animals, we did 5 allotransplants (donor heart from another dog) and 1 autotransplant (heart was removed from a dog and then re-implanted). Unfortunately, we were unable to get long term survival of any allotransplant animals, with most of them dying from surgical complications related to bleeding at the anastomosis sites. We did have a 36 hour survivor with a closed chest, which was considerably better than the University of Rochester experience, and we demonstrated several cases of excellent cardiac function and good blood pressures after overnight storage of hearts. However, we were not able to overcome the surgical problems, which appeared technical.

In support of this hypothesis, we did have a long-term survivor (still doing well after more than 12 months) and this was the autotransplant dog. However, this animal had not quite been a full autotransplant, as all vessels but the aorta had been transected, but the aorta itself had been left intact. A number of the allograft dogs had died of aortic bleeding, which we presume is related to the extreme thinness of the aorta in dogs, which is one of the parts of dog anatomy which is most unlike humans. Possibly our other dogs died not because of the donor heart, but because they simply did not survive the aortic anastomosis problems which the autotransplant dog avoided. However, the autotransplant dog also had the least heart ischemia time, and we cannot rule out some unexpected consequence of this.

At present, we report this project is on hold until we see if a goat model is available at another site. We still believe this project is doable in theory with dogs, and doing it has given us a great deal of experience in acute dog intensive care. However, our animal use committee has recommended that we look into other models for the time being.

PROJECT 4: Dog Vivarium and Model Development, Breeding Program, and Community Outreach.

Our dependence on outside "class B" dog dealers as a source of experimental animals was ended in 2001, as we began a program of complete in-house breeding of dogs, which were to be raised from puppies through adulthood at our facilities. This continues, and we still maintain about 60 dogs in all stages of development, in complete indoor controlled environment kennel facilities overseen by a veterinarian and regularly inspected by the USDA. Our breeding program has generally been successful (we have a recent Labrador stud dog used as both pet and guard dog—but never experimental dog-- to bring outside blood into the colony. We have had good relations with the local veterinarians. This is partly as a result of using our colony as a source of donated dog blood to save injured dogs in a local vet practice. This does not harm our colony, and makes for better public relations. We benefit also from having our inspecting vet take time on his inspection days to do cat and kitten spay and neutering for a local cat adoption group. This benefits both the vet and the local cat-advocacy group, which knows that we don't use cats in any of our experimental programs.

Our last 3 evaluations from our USDA inspector were nominal, with no problems found by the inspector at all. This series of inspections with no problems and a perfect record is necessary for us to formally apply for Office of Laboratory Animal Welfare (OLAW) certification for our site, which we are now doing.

SUMMARY. Although we have had difficulty in using the dog as a heart transplant model, the dog remains a particularly valuable animal for the testing of deep hypothermia, resuscitation and brain-cooling strategies by means of the lungs.

In particular, the dog remains a valuable model because if its' ease of clinical evaluation. A standard stethoscope exam of a dog's chest reveals details of obstructive lung problems which cannot be evaluated in rodent or lamb models. Perfluorcarbon lung lavage results

in almost no detectable decrease in airway patency in the lungs, but if done incorrectly, or with the wrong fluorocarbon, severe problems begin to emerge at 24-48 hours. Due to the difficulty and expense in maintaining a research dog colony, no other group we are aware of, is studying lung lavage in dogs (very few groups still use the dog model for any research at all). Other research groups studying lung lavage have used rodents, fetal lambs, or pigs, and none have been allowed to survive the acute experiment. Thus, other groups have not discovered the long term effects which we have discovered and are well on the way to solving. However, all the long term problems will need to be worked through and understood in an animal model, before being applied to humans.

We are particularly pleased that Lampe and Becker, resuscitation researchers at Argonne National Laboratories, had not been able to see in 2007, even in theory, how rapid brain-cooling in the range we now routinely achieve, might be accomplished. This accomplishment has been due to the very effective coordination between LEF funded institutions in development of novel technology, over many years.

Steven B. Harris, MD
Prepared for Critical Care Research

Brief Annual Report for Critical Care Research for

2008

Notes Prepared For Saul Kent and Bill Faloon
by Steve Harris, Sandra Russell, Joan O'Farrell.

PROGRESS IN CALENDER YEAR 2008

Critical Care Research made major progress in 2008, finally reaching our goal of brain cooling at -1 C/min with a patentable device. The final patent application was submitted at the end of 2008.

Chief Projects.

[1] Work on Optimal Liquid Lavage Cooling Strategy, with extremely rapid cooling rates achieved. This work has been thought to be difficult to achieve by others, even in theory.

[2] Summary of Development State and Theory for the Recently Submitted Cooling Device Patent: Work on the first Truly Portable High Capacity Lung Lavage Device, with device patent applied for, late September 2008 (less than one year after a preliminary patent application detailing high rates of cooling by this was submitted to the World Patent Office).

[3] Continued Dog Breeding and Experimental Dog Vivarium Management.

SUMMARY PROGRESS

PROJECT 1: Work on Optimal Liquid Lavage Cooling Strategy, with a strategy developed for extremely rapid cooling rates.

In previous experiments we have found that cold perfourocarbon lung lavage in dogs applied in a non-injurious manner consistently delivers at least 0.1 C/min cooling rate to the brain early in cooling (i.e, with at least 30 degrees °C temperature difference between cooling fluid and brain temperature), and that the maximal cooling rate may be as high as 0.5 °C/min (see Harris, S.B. Darwin, M.G., Russell, S.R., O'Farrell, Fletcher, Mike, and Wowk, Brian, Rapid (0.5°C/min) minimally invasive induction of hypothermia using cold perfluorochemical lung lavage in dogs. *RESUSCITATION* 50: 189-204, 2001. PMID: 11719148). This cooling rate was achieved with a type of lung lavage which was a-synchronous with breathing, due to the requirement for gas ventilation at least once

every 15 seconds, and the difficulty in complete delivering and removing a large bolus of cold perflurocarbon in this time. One the other hand, small boluses of fluorocarbon were found to transfer heat less efficiently in our model. For example, the maximal cooling rate achieved in this paper was achieved at a perfluorcarbon lavage rate of 31 mL/kg/min, but this required a lavage time of 37 seconds. The lavage volume for this maximum was 20 mL/kg, but when this was dropped to 9 mL/kg (allowing a lavage time of 16 seconds) the heat transfer efficiency dropped from 60% to 40%, and consequently cooling rates dropped by 30%, even though the lavage rate had increased slightly to 36 mL/kg/min. At the time, we suspected that we had reached a limit, which was due to diminishing heat transfer efficiency, and mechanical efficiency of removal, with small lavage volumes.

In subsequent work, however (detailed in our previous reports) we found that the drop in heat transfer efficiency was avoidable if perflourocarbon infusion was synchronized with oxygen gas insufflation (best provided by hand bag-valve unit), so that cold perfluorocarbon could always be provided into lungs being expanded by insufflation of pure oxygen gas. We were able to show heat transfer efficiencies as high as 50%, even with lavage volumes as low as 3 mL/kg, with this technique. However, limitations in our equipment (primarily in suction efficiency) still prevented us from conducting lavage faster than 20 mL/kg/min at these small lavage volumes. From theoretical considerations, we understood that we would need total lavage rates of about 60 mL/kg/min to achieve brain cooling rates in the neighborhood of > 1 C/min.

Challenge of Becker, et al. In 2007, Lance B. Becker and Joshua W. Lampe published a paper in Expert Rev. Med. Devices 4(4), 441-446 (2007) in which they challenged experimenters to design a cooling system which would lower brain temperature by 4°C in the first 5 minutes of applications. They wrote:

"The induction of mild hypothermia, lowering body temperature by 4°C, is gaining acceptance as an acute therapy for the treatment of hypoxia and ischemia following cardiac arrest and many life-threatening injuries. When hypothermia is used following ischemia (as opposed to before ischemia), it needs to be performed rapidly for the greatest benefit, preferably within 5 min. A simple model reveals that this poses a significant bioengineering challenge as the rate of heat transfer is severely limited, owing to a relatively confined fundamental parameter space. Current methods of cooling include external cooling devices, such as cooling blankets or ice bags, which are simple to use, relatively inexpensive but slow. Internal cooling has the best ability to cool more rapidly but current devices are more invasive, costly and most are still not able to provide cooling within the rapid 5-min interval."

Lampe and Becker go on to a discussion of theoretical amounts of heat which can be removed by a number of methods, including cardiac bypass, but note that this takes at least 1 hour to implement. They conclude:

"Hypothermia of 3-4°C must be achieved within 5 min for maximum protection when required after a period of ischemia. The human body is well designed to prevent heat loss, creating a significant bioengineering challenge in a relatively confined fundamental

parameter space. The minimum temperature and the maximum volume of coolant that can be introduced into the body are significant engineering obstacles."

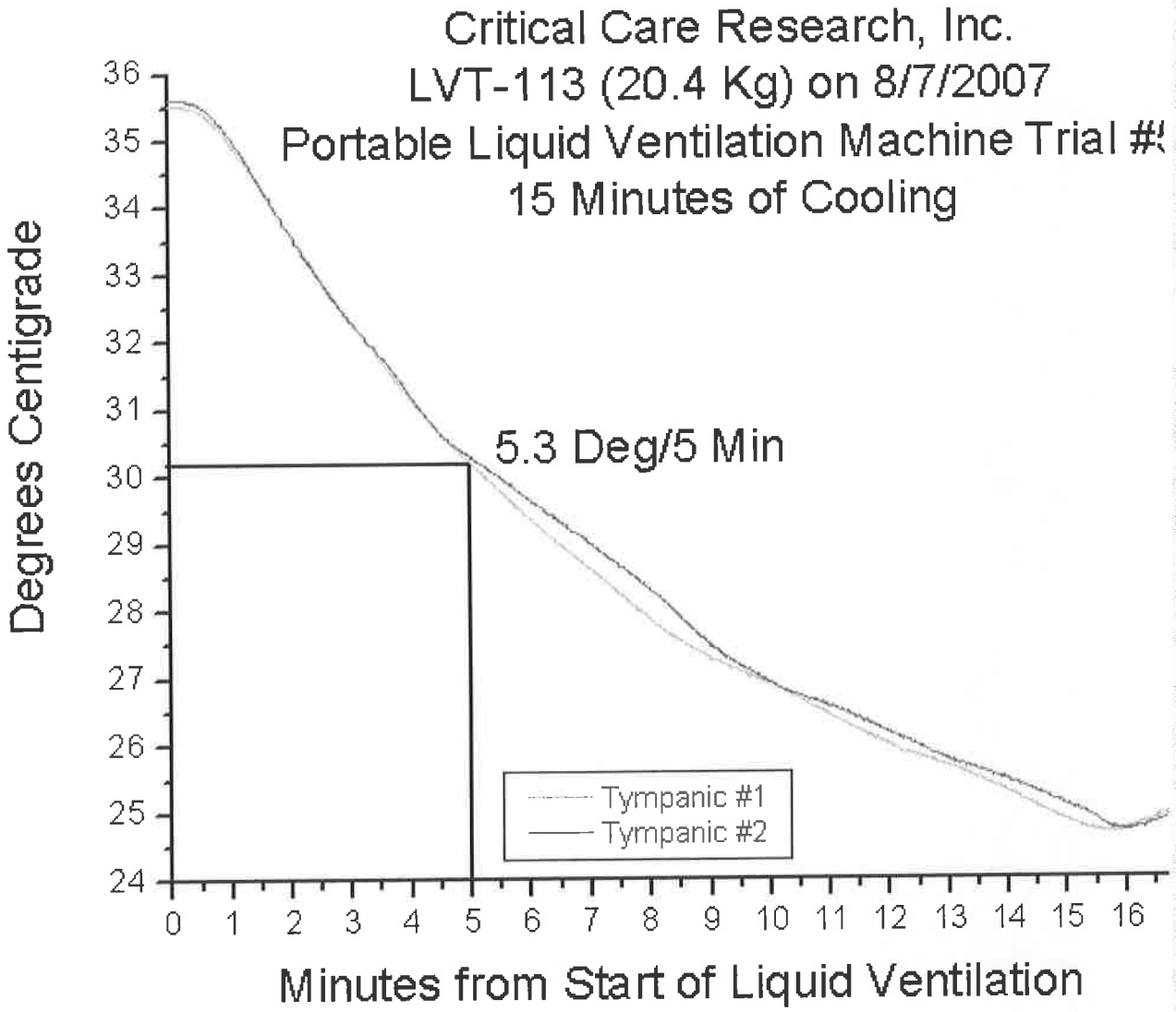
In reading Lampe and Becker's article we realized that they seriously miscalculated the amount of heat which would be need to be removed during rapid cooling, due to their inexperience with the decrease in effective heat capacity of the body, which occurs during very rapid cooling of the blood. Our previous experiments had found that only a 70% "core" mass (which includes the brain, fortunately) is cooled in the first few minutes of cooling, and that if cooling continues past 5 minutes, the extra heat to be extracted in the 6th minute will make up for the "re-equilibration" which takes place within the animal or human, as the cold core and brain are re-warmed by the periphery, which does not initially cool as fast as they do. Thus, we knew that Lampe and Becker's estimates of needed heat extraction were off by a factor of 2, and also that their estimations of the amount of cold fluorocarbon which could be delivered to the lungs, was incorrect.

In our last report to the Life Extension Foundation, we detailed problems we had had with the breadboard prototype lung lavage machine using peristaltic pumps (ie, "roller-type" pumps which squeezed flexible tubing, as in a traditional heart-lung machine (these machines are now beginning to use centrifugal pumps, which cannot be used for our purposes, due to their incompatibility with air). Although our early peristaltic type pumps were adequate for fluorocarbon suction they were not able to provide the very high suction rates which were required to return 60 ml/kg/min of PFC, which we estimated would be the minimum to achieve Lampe and Becker's goal of -4 C in 5 minutes. The major reason for this was the failure of these pumps to be able to return not only the 60 60 ml/kg/min of PFC, but also the volumes of air from the lungs, which amounted to several times this volume, and which had to be removed before the lungs collapsed to the point of being able to remove liquid.

Work on Portable High Capacity Lung Lavage Device. Accordingly, beginning in 2007 we contracted with Gary Battiano and Charles Platt at Suspended Animation, Inc. (also funded by the Life Extension Foundation) to provide a suitable high capacity (high suction) machine, which would be completely portable (able to run from batteries) and also able to cool more than -4 C, should this be required. In designing this machine we made use of the high-capacity commercial "plate-type" heat exchangers described in our last report (capable of nearly 600 watts/degree heat transfer between ice-water and cold perfluorcarbon). Battiano and Platt also discarded the bulky peristaltic pumps and replaced them with inexpensive commercial fluid diaphragm pumps, which proved far superior, and able to run on low voltage D.C.

With the superior suction capabilities of this device, combined with its ability to provide a constant supply of perfluorocarbon cooled to 2 °C (from a reservoir containing only 7 liters of fluid) we performed a series of 30 lung-lavage dog experiments in 2007 and mid 2008. The first results were encouraging enough to apply for a preliminary patent on the device in October 2007, and a full patent application was filed (Platt, Battiano, Harris) for the device in October, 2008.

A graph from one of the early experiments with the device, after design parameters had been partly adjusted, illustrates performance:



This graph shows that Lampe and Becker's stated goal (which they thought impossible without a direct application of a phase-change substance to the body) of -5 °C in 5 minutes has been exceeded with the device. In addition, in this experiment, we were able to cool the animal's brain and heart to 25°C (77 °F), in just 16 minutes.

Later experiments with the device show that 6-7 minutes of application of 60 mL/kg/min lung-lavage with ice-cold perfluorcarbon (2 °C) results in at least -4 °C drop in brain

temperature in the first 5 minutes, and that application of lavage for more than 6 minutes results in a permanent body core temperature of -4 °C. All of these results are novel, and should be of great importance to the resuscitation community.

In numerous repeatable experiments we found that it requires 6 minutes of lung lavage to result in a permanent -4 °C temperature drop, as well as an instantaneous drop of -4 C in the first 5 minutes of lavage. This is due to the fact that the first minute of lung lavage results in almost no measured brain cooling, due to heat exchange delays while the blood is cooled via the lungs, and the "cold" is transferred to the brain. However, when lavage is stopped, cooling of the brain continues for nearly a minute while the blood volume and the lungs (which may be as much as 2.5 °C colder than the brain, or in other words, at 29 °C when the brain is at 32.5 °C).

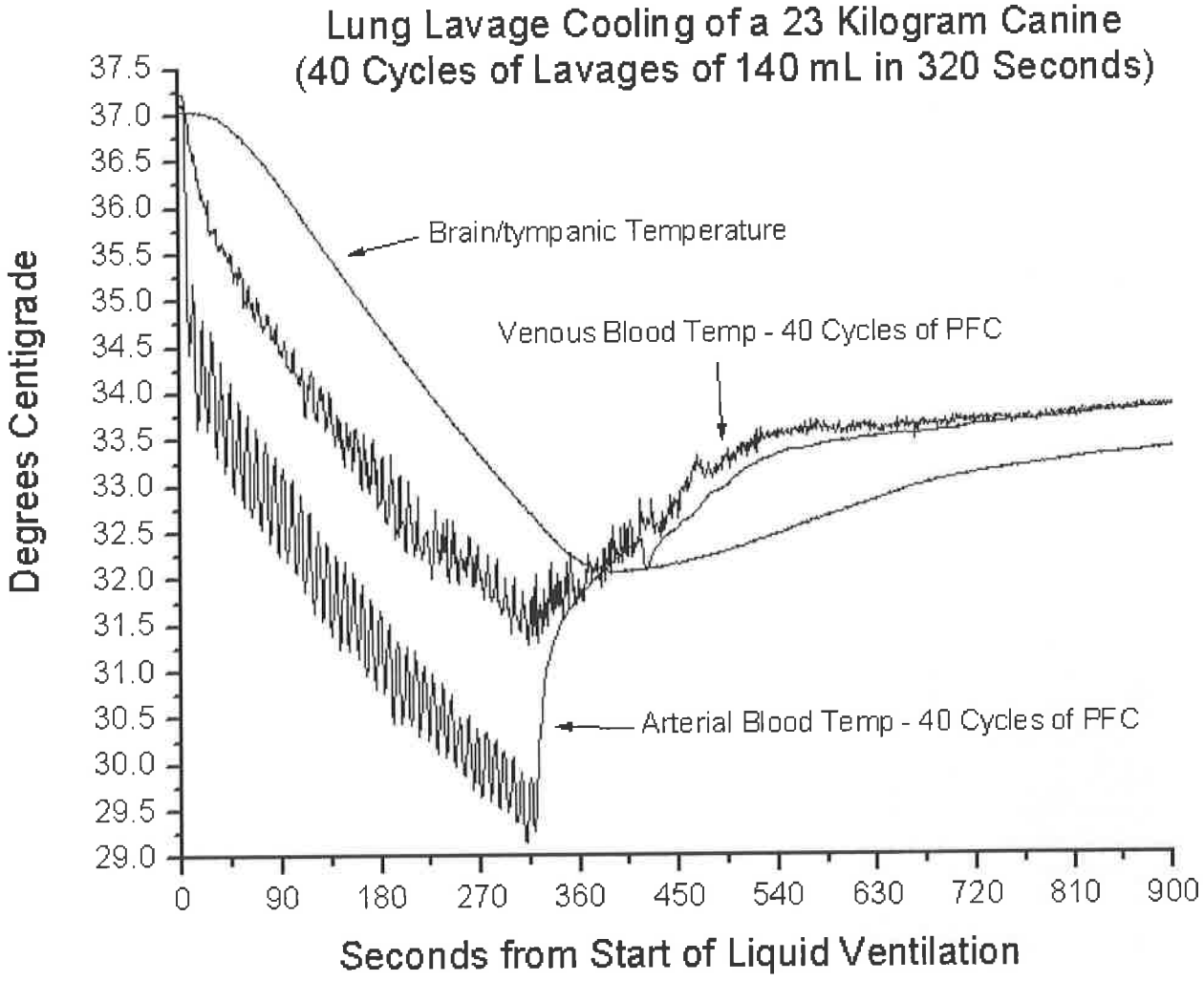


Figure: Animal Lavaged with Device in Patent, 2008. In the Figure above temperatures in the venous system, arterial system, and brain are recorded. Lung lavage is given for 5.3 minutes. Brain temperature does not begin to drop rapidly measurably until 45 seconds, which reflects a circulation delay, as the cooling of the arterial blood is seen to be immediate. However, the overshoot effect occurs after the procedure is stopped, and the minimal brain temperature does not occur until almost 6.75 minutes (a minute and a half after the procedure has stopped), and it is 32.2 °C, which is -4.8 drop from a beginning temperature of 37 °C (98.6 F). Over the next hour the brain will warm to 33.7 °C (-3.3 °C), as the cold core of the body equilibrates with the warmer exterior (about 20% of the total thermal "mass"). In general, continuous lavage of about 6 minutes is required to guarantee a -4 °C drop at 5 minutes, as well as a permanent brain temperature drop of -4 C even after body and brain equilibration, one hour after liquid ventilation cooling has been stopped. To cool the body and brain permanently by -4 °C requires an early and instantaneous brain cooling of about -5.5 °C, which later develops into a permanent "whole body" drop of -4 °C after equilibration of the cold thermal core with the warmer (but less well perfused) periphery. Thus, the brain is rapidly cooled by -5.5 °C to retain -4 °C.

Project #2: Summary of Development State and Theory for the Recently Submitted Cooling Device Patent: Extensive canine experiments were conducted by the inventors in order to ascertain the most effective and safest manner in which canine core temperatures could be reduced by cycling perfluorocarbon, as the biocompatible liquid, into and out of the lungs. In these experiments it was demonstrated that lung lavage with cold perfluorocarbon transferred the maximal amount of heat from the lungs of the animal on a timescale of at least as fast as the lavage could be administered and withdrawn, up to rates of at least 50 mL per kilogram of canine body weight per minute. In these experiments no waiting time was needed between the time the lavage was delivered into the lungs, and the time it was removed. It was also shown that only a fraction of the thermal content of the lavage, typically about 50%, equilibrated with the animal, but that this fraction was very little influenced by residence time in the lung, on a time scale of a few seconds to a few tens of seconds, which was typical of delivery and removal time of a lavage. For these reasons, it was thought that maximal heat transfer over time took place without any residence time between the delivery and removal of lavage volumes, with the lavage removed from the lung as quickly as possible after being introduced.

These experiments, however, were done using a method which that did not coordinate gas ventilation and liquid lavage. One reason for this was because of severe constraints in how fast lung lavage with perfluorocarbon could be delivered and removed using the previously available peristaltic devices.

Also, it was also thought, incorrectly, that lavage volumes would be required to be several times the amount of mechanical dead space in the dog respiratory system (i.e., several times a measured dead space of 6 mL/kg) in order to minimize the "thermal dead space" which was seen when small volumes of perfluorocarbon (on the order of 9 mL/kg

or less) did not transfer heat as efficiently as larger lavages (20 mL/kg). Only when a series of experiments using lavage volumes as small as 3 mL/kg demonstrated a heat exchange that was comparable to the higher lavage volumes per weight of the animal, was it realized that proper coordination of lavage and gas ventilation could effectively transfer heat from smaller infusion volumes than the theoretical dead space volume.

We believe that the reason was due to an increased efficiency in liquid removal with the correct type of suctioning, coupled with turbulence in the delivered and removed liquid. Such turbulence corresponds, in terms of heat transfer, to the familiar elimination of dead space by "high frequency (oscillating) ventilation" or "panting" in the mechanics of ventilatory mass (gas) transfer. In short, if the perfluorocarbon was delivered to and removed from the lungs quickly enough, a volume of liquid lavage was required that was much smaller than anatomical dead space in the lungs.

At the same time, a number of ways of delivering gas ventilation to the lungs were tried. As it was apparent that with the small volumes of perfluorocarbon being used (as small as 3 mL/kg) that coordinated gas ventilation (normally 10 mL/kg per breath) could and would supply most of the gas exchange, then the key question was how to supply the quantity of lung gas ventilation that would be required to keep the CO2 levels in the animal's blood at normal levels. If the gas used was pure oxygen, it was found that CO2 removal was the limiting factor in ventilation. CO2 removal is much more sensitive to low gas ventilation volumes than oxygen level in these circumstances, just as it is with total liquid ventilation. This occurs because at the low levels of CO2 (4 to 5 % or 40 mmHg partial pressure) which occur in normal expired gas, the amount of CO2 in a volume of either gas or perfluorocarbon is always small, when compared to the amount of oxygen contained in ventilatory gas or liquid if 100% oxygen is used.

We also found in a series of experiments that about 100 mL/kg/min of gas ventilation per minute alone was needed to normalize CO2 in anesthetized 20-25 kg dogs. This could be delivered in as few as 4 breaths/min of 25 mL/kg for each breath, but slower rates required breath volumes which resulted in unacceptable ventilatory pressures (>25 cm H20) when liquid was present in the lungs. Also, we found that 100 mL/kg/min of gas(oxygen) ventilation was not quite sufficient to maintain normal pCO2 during liquid ventilation, and pCO2 rose to 50 to 60 mmHg after 18 minutes of lavage, even with small (3 mL/kg) lavages.

A series of coordinated experiments with 3 mL/kg perfluorocarbon lavage and 25 mL/kg gas ventilation was initiated and found to give efficient heat transfer, but the relatively slow liquid lavage rate (3 mL/kg x 4 lavages/min = 12 mL/kg/min) resulted in relatively slow rates of cooling of -0.25 C/min. However, the rate of perfluorocarbon return available with the type of device being used (the peristaltic pumps) limited the lavage rate to 12 mL/kg/min for this size animal. In the MMLV patent and later publications we described cooling rates up to minus 0.5 C°/min with larger lavage rates (liquid ventilation rates) up to 36 mL/min. However, this rate of lavage required relatively large infusions of 19 mL/kg, in order to take advantage of the rapid return suction of infusion liquid which is possible when the liquid contains few gas bubbles (as happens with large lavage

volumes). This is because liquid without bubbles is very much easier to pump or suction.

However, this rapid return was not possible with the peristaltic device with small lavage volumes, or with subsequent devices, until the implementation of a better machine using diaphragm pumps increased suction efficiency. The best pumps for the purpose turned out to be relatively inexpensive marine bilge pumps, such as are used in the small boat industry. These also have the advantage of working on low voltage DC, and are directly compatible with nickel metal hydride batteries.

Large lavage volumes of 20 mL/kg as described in the previous 2001 MMLV (Mixed Mode Liquid Ventilation) patent also required a relatively slow infusion delivery due to the size of the lavage (1.6 lavages/min), and thus discoordination of lavage and gas ventilation in time (since respiration must be done much faster than 1.6 breaths per minute).

With the availability of rapid lavage liquid suction in our new diaphragm pump apparatus, it became possible not only to coordinate gas ventilation to lavage, but also to use relatively small lavages of 6 mL/kg with large amounts of gas (20 to 25 ml/kg), yet remove and infuse them sufficiently rapidly to perform 7.5 lavages/minute and 7.5 gas breaths per minute. This resulted in a liquid lavage rate of about 6 x 7.5 mL = 45 mL/minute, and cooling rates of approximately 1 C/min.

Since efficiency was maintained, the factor of 4 increase in effective lavage rate resulted in about the same factor of 4 improvement in cooling rate over the coordinated breath/lavage dogs which received 12 mL/kg/min of perfluorocarbon. In addition, ability to perform 7.5 lavages per minute offered the opportunity of performing 7.5 gas breaths of 500 mL per minute (3750 mL/min oxygen total), which in a 25 kg dog is 150 mL//kg/min gas ventilation. This increase was enough to offset the diffusion barrier seen for CO2 in liquid ventilation, and to result in normal levels of CO2 of 40-45 mmHg during liquid lavage.

In turn, this allowed the continuing novelty of previous designs, which use no CO2 liquid scrubber or perflourocarbon oxygenator. The volumes of perfluorocarbon are kept low, and this allows the lungs to perform not only oxygenation and CO2 removal for the animal, but also for the volume of perflourocarbon maintained in the cold perflourocarbon reservoir. This greatly simplifies design of the device.

With loss of the constraint of a minimal lavage volume needed for good efficiency of heat transfer, it proved possible to coordinate smaller liquid lavages at effective breathing rates. At the same time, a series of experiments showed that small lavages of perfluorocarbon fluid, of about the FRC in volume, transferred heat maximally quickly, with the least increase in pressure and the least damage to the lung, when the lavages were administered as the lung was being simultaneously inflated by a breathing gas, preferably with 100% oxygen, as the lavage fluid was being introduced simultaneously.

Less pressure was required to inflate the lungs, if the inflation volume was a mixture of

gas and liquid, than if the volume was liquid alone, presumably because simultaneously introduced gas is able to find, and recruit, non-dependent volumes of the lung which are not accessed by the much heavier liquid. Furthermore, it was found that heat transfer is more efficient in the dorsal recumbent dog than the dog in the lateral or ventral recumbent (prone) positions, presumably due to the larger surface area of dependent lung available to a heavy liquid, in a dorsally recumbent animal.

A number of commercial perfluorocarbons were tried for these experiments, and Fluorinert™ liquid FC-84 (perfluoroheptane) and Fluorinert™ liquid FC-40 (perfluorotributylamine) from 3M™ were both found to be acceptable liquids for use as the biocompatible liquid used in the experiments. Commercial Perflubron™ is not suitable for liquid lavage at the liquid temperatures used in the experiments because it freezes at 4°C and is too viscous to be useable below 15° C.

An additional series of experiments showed that delivery of cold perfluorocarbon directly into the major tracheal branches of lung with small (12 F) catheters, followed by distal removal of liquid in from these catheters, or even distal infusion of fluid, followed by removal from a single catheter in the upper trachea, did not increase the efficiency of heat transfer of lavages. At net rates of lavage of 12 mL/kg/minute of perfluorocarbon (infusion rate 60 mL/kg/min, fluid suction rates up to 25 mL/kg/min), efficiency of heat exchange did not rise above 60% (Abstract poster presented at Society for Critical Care Research meeting, 2002). However, these experiments did show that dogs could be cooled by -3°C in less than 30 minutes (this is about 6 times faster than commercial intervenous coolers for human clinical application).

The relatively slow cooling rate in the above experiments (0.1 C/min) could have been doubled by maximally chilling infused perfluorocarbon to 1-2° C, but a further limit at 0.2° C/min was caused by the relatively small rates of absolute suction which can be applied though small tubes (500 mL/min absolute). This contrasts with the 2 to 3 L/min suction which can be obtained for liquid from conventional flatwire venous drainage cannulae, such as the 17 F Biomedicus™ brand canulae used for surgical femoral artery bypass.

EXPERIMENTS WITH DIFFERENT CANNULAE FOR DELIVERY AND SUCTION. Furthermore, it was found that high speed jet delivery of cold perfluorocarbon to the distal ends of the trachea caused evidence of damage, as hemorrhage was seen in the trachea on necropsy at 24 hours, corresponding to the tip ends of the 12 French catheters. This damage disappeared when perfluorocarbon was merely introduced into the upper end of the endotracheal tube. In this case, to prevent perfluorocarbon overflow, the lung was merely required to be inflated with oxygen gas ahead of the perfluorocarbon. When this was done, a flow of cold perfluorocarbon that was introduced into the top of the endotracheal tube dropped into the lungs and was further spread by an insufflated breath of oxygen into the interior sections of lungs where heat exchange took place.

DEAD SPACE. In a similar fashion, attempts to minimize fluid dead space in the lungs

by putting small suction catheters at the ends of the bronchi where not ultimately successful as methods of increasing net rate of heat transfer. This was, in part, due to the fact that the small diameter of the catheters limited the rate at which fluid could be removed from the lungs, and this limitation proved to further limit the rate of heat transfer, because it limited rate of liquid transfer. Eventually, in suction, it was found that the single greatest assistance to time-efficient removal of fluids from the lungs, and thus in time efficient transfer of heat, lay in application of gentle negative pressure so that the lungs were collapsed, as at the end of a forced exhalation. This made maximal fluid from the lungs available, as at the end of a squeezed sponge, and this fluid could be picked up at the end of a normal endotracheal tube, situated relatively high up in the trachea, and carried out by suction. Thus, the suction catheters of previous models of machine were eliminated, and with them, the limitation in suction rates caused by their smaller diameter.

In summary, we realized that a device which introduced fluid to the top of an endotracheal tube at the same time a gas breath was applied, and then removed both gas and liquid from the top of the tube while suction was applied to the entire cuffed tube, adequately performed both the job of administration and removal of liquid from the lungs. No second luminal tube, as our prior devices have used (and all other experimenters, to our knowledge, have used) are needed.

By this reasoning, and with significant empirical experimentation, a time-efficient technique for maximal heat transfer from small lavages of perfluorocarbon within the lung of a canine was eventually developed, and implemented. The last portable heat exchange apparatus developed under our direction, recently submitted for patent, uses all these systems for maximal rate of heat transfer.

Animals subjected to only -4°C of cooling (enough to trigger post-resuscitation brain protection, according to many other studies) do particular well in this model. When allowed to survive for the long term, they at first have minimal symptoms (slight airway obstruction) which is not severe enough to keep them from walking and eating. Thereafter they recover completely, after the first week. In animals which we have sacrificed at 48 hours to examine lung pathology, we see minimal or no hemorrhage indicative of pressure-damage. We are able to keep total lung pressures low (< 30 cm H20) in this model, mainly by keeping lavage volumes to below 9 mL/kg (the oxygen gas inspiration volume is about twice as much, or 20-25 mL/kg). Currently we are routinely running 8-9 mL/kg lavages at 8 seconds/lavage (7.5 breaths and lavages/minute), for total fluorocarbon lavage rates of 60-68 mL/kg/min.

The rates of cooling we are seeing (- 1 C/min) are comparable to cardiac bypass cooling rates for the brain. Yet they do not require any kind of surgical preparation.

The dog remains a particularly valuable animal for the testing of deep hypothermia, resuscitation and brain-cooling strategies by means of the lungs. In particular, the dog remains a valuable model because if its' ease of clinical evaluation. A standard stethoscope exam of a dog's chest reveals details of obstructive lung problems which

cannot be evaluated in rodent or lamb models. Perfluorcarbon lung lavage results in almost no detectable decrease in airway patency in the lungs, but if done incorrectly, or with the wrong fluorocarbon, severe problems begin to emerge at 24-48 hours. Due to the difficulty and expense in maintaining a research dog colony, no other group we are aware of, is studying lung lavage in dogs (very few groups still use the dog model for any research at all). Other research groups studying lung lavage have used rodents, fetal lambs, or pigs, and none have been allowed to survive the acute experiment. Thus, other groups have not discovered the long term effects which we have discovered and are well on the way to solving. However, all the long term problems will need to be worked through and understood in an animal model, before being applied to humans.

We are particularly pleased that Lampe and Becker, resuscitation researchers at Argonne National Laboratories, had not been able to see, in 2007, even in theory, how rapid brain-cooling in the range we now routinely achieve, might be accomplished. This accomplishment has been due to the very effective coordination between LEF funded institutions in development of novel technology, over many years.

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Our dependence on outside "class B" dog dealers as a source of experimental animals was ended in 2001, as we began a program of complete in-house breeding of dogs, which were to be raised from puppies through adulthood at our facilities. This continues, and we still maintain about 60 dogs in all stages of development, in complete indoor controlled environment kennel facilities overseen by a veterinarian and regularly inspected by the USDA. Our breeding program has generally been successful (we have a recent Labrador stud dog used as both pet and guard dog—but never experimental dog-- to bring outside blood into the colony. We have had good relations with the local veterinarians. This is partly as a result of using our colony as a source of donated dog blood to save injured dogs in a local vet practice. This does not harm our colony, and makes for better public relations. We benefit also from having our inspecting vet take time on his inspection days to do cat and kitten spay and neutering for a local cat adoption group. This benefits both the vet and the local cat-advocacy group (we don't use cats in any of our experimental programs, but our knowledge with cats has been handy in suspensions of cats from cryonicists).

All of our last evaluations from our USDA inspector have been perfect, with no problems found by the inspector at all. This series of inspections with no problems and a perfect record is necessary for us to formally apply for Office of Laboratory Animal Welfare (OLAW) certification for our site.

We continue to provide upgrades which have caused our USDA inspect to use our kennel as a model for other kennels he works with. These include behavioral modulation programs for our dogs, which results in a nearly noise-free colony, instead of continuously barking animals.

We have also been successful recently in covering the floors of our kennels with epoxy-resin of the type which is used to provide a non-slip surface over concrete in garages. In concert with stainless steel cages and plastic-covered pallets, this covering allows for non-slip footing for the animals in their daily trips to their outdoor run and wading pool area, while allowing the kennel area to still be cleanable with a high temperature steam pressure washers. All of these systems are far beyond what is required for kennel husbandry of dogs by the USDA, and are being watched with interest and satisfaction by our animal inspector.

We have one of the few working colonies of research dogs left in the country. Other programs which are forced to rely in liquid ventilation models in rodents or fetal lambs, are not able to do long term studies, and thus never seen the beginning lung injury which we have learned to prevent at 24-48 hours post-ventilation. We believe that our model will only be successful elsewhere with swine or sheep, if at all, but both species are extremely difficult to monitor for brain temperature and for clinical distress in respiratory experiments (such as wheezing in lung experiments—a prime symptom of damage for us). We believe that our colony provides a unique one in which rapid induction of hypothermia by this method will be developed, or else not at all.

Steven B. Harris, MD
Prepared for Critical Care Research

Brief Annual Report for Critical Care Research for

2010

Notes Prepared For Saul Kent and Bill Faloon
by Steve Harris, Sandra Russell, Joan O'Farrell.

PROGRESS IN CALENDER YEAR 2009

Critical Care Research made progress in 2010, attempting to explore our goal of brain cooling at -1 C/min with a patentable device, without major lung damage. A second version of the device was finished in 2010, designed by Charles Platt and further tested by us in the later part of the year, and in to 2011. The new machine was found to perform excellently, leading to extremely uniform results.

Some setbacks occurred relative to obtaining the correct fluorocarbons for lung lavage, as 3M Corporation discontinued a primary working fluorocarbon, FC-84. The search for a new fluorocarbon to replace FC-84 in our mixture has begun.

A review of the literature shows that lung lavage is being used to cool animal in both the pig and rabbit models, and that it is successful in cooling the heart and brain in resuscitation in pigs. However, the dog continues to be the correct model for lung lavage, since pig lungs have been found by others to react strongly to fluorocarbon even an hour after lavage, and so pig models have not been used as survival models. By contrast, rabbits are too small to assess lung damage during life.

Chief Projects.

[1] Test of liquid lavage cooling efficiency by direct methods, with high cooling rates. Literature review done for USDA

[2] Continued experimental dog vivarium management.

SUMMARY PROGRESS

PROJECT [1] Test of Liquid Lavage Cooling Efficiency by direct methods, with high cooling rates. In previous experiments we have found that cold perfluorocarbon lung lavage in dogs applied in a non-injurious manner consistently delivers at least 0.1 C/min cooling rate to the brain early in cooling (i.e., with at least 30 degrees °C temperature difference between cooling fluid and brain temperature), and that the maximal cooling rate may be as high as 0.5 °C/min (see Harris, S.B. Darwin, M.G., Russell, S.R., O'Farrell, Fletcher, Mike, and Wowk, Brian, Rapid (0.5°C/min) minimally invasive induction of hypothermia using cold perfluorochemical lung lavage in dogs.

RESUSCITATION 50: 189-204, 2001. PMID: 11719148). This cooling rate was achieved with a type of lung lavage which was a-synchronous with breathing, due to the requirement for gas ventilation at least once every 15 seconds, and the difficulty in complete delivering and removing a large bolus of cold perfluorocarbon in this time. One the other hand, small boluses of fluorocarbon were found to transfer heat less efficiently in our model. For example, the maximal cooling rate achieved in this paper was achieved at a perfluorcarbon lavage rate of 31 mL/kg/min, but this required a lavage time of 37 seconds. The lavage volume for this maximum was 20 mL/kg, but when this was dropped to 9 mL/kg (allowing a lavage time of 16 seconds) the heat transfer efficiency dropped from 60% to 40%, and consequently cooling rates dropped by 30%, even though the lavage rate had increased slightly to 36 mL/kg/min. At the time, we suspected that we had reached a limit, which was due to diminishing heat transfer efficiency, and mechanical efficiency of removal, with small lavage volumes.

In subsequent work, however (detailed in our previous reports) we found that the drop in heat transfer efficiency was avoidable if perfluorocarbon infusion was synchronized with oxygen gas insufflation (best provided by hand bag-valve unit), so that cold perfluorocarbon could always be provided into lungs being expanded by insufflation of pure oxygen gas. We were able to show heat transfer efficiencies as high as 50%, even with lavage volumes as low as 3 mL/kg, with this technique. However, limitations in our equipment (primarily in suction efficiency) still prevented us from conducting lavage faster than 20 mL/kg/min at these small lavage volumes. From theoretical considerations, we understood that we would need total lavage rates of about 60 mL/kg/min to achieve brain cooling rates in the neighborhood of > 1 C/min.

Work on Portable High Capacity Lung Lavage Device. Accordingly, beginning in 2007 we contracted with Gary Battiano and Charles Platt at Suspended Animation, Inc. (also funded by the Life Extension Foundation) to provide a suitable high capacity (high suction) machine, which would be completely portable (able to run from batteries) and also able to cool more than -4 C, should this be required.

With the superior suction capabilities of this device, combined with its ability to provide a constant supply of perfluorocarbon cooled to 1 °C (from a reservoir containing only 7 liters of fluid) we performed a series of lung-lavage dog experiments in 2010, using a second copy of this device, which had been improved by Platt to provide for ease in recovering fluorocarbon.

Experiments with the device are possible with collection of the fluid from each lung lavage, which is collected, measured in volume in a separate insulated container, and then analyzed in temperature. This data gives us data for heat transfer that can be estimated directly from the fluid itself, instead of having to infer it from the dog's estimated heat capacity and degree of cooling. With this device we can calculate the heat removed directly and accurately. This could not be done in measuring fluid temperature during suction (as we discovered) because the temperature and volumes change too fast for a good integration.

A new series of experiments shows that heat transfer to the dog with lavages of 6 ml/kg and 6.5 mL/kg every 7.5 seconds (the maximum that was compatible with little lung damage) gave a heat transfer efficiency of almost exactly 60%.

With the new device we found that 7 minutes of application of 52 mL/kg/min lung-lavage with ice-cold perfluorcarbon (1 °C) results in almost -4 °C drop in brain temperature in the first 5 minutes, and that application of lavage for more than 7 minutes results in a permanent body core temperature of -4 °C (the temporary drop in brain temperature at 10 minutes is almost -6 °C, but this soon equilibrates). The amount of total fluid necessary to cool the entire animal by -4 °C was a consistent 340 mL/kg, or 85 mL/kg/°C. This amount of fluid now sets the standard of ice cold fluorocarbon which is necessary to cycle through the lungs in order to achieve classical "mild hypothermia" for resuscitation. We believe it will also be the amount of fluid necessary per degree of permanent brain cooling in humans.

This cooling corresponds to a final heat capacity of the animal of 0.7 kcal/kg/°C (the same as in humans) with a heat extraction efficiency of 60%. Direct calculation has given us an average extraction efficiency of 62%, making the heat capacity of our average animal slightly higher.

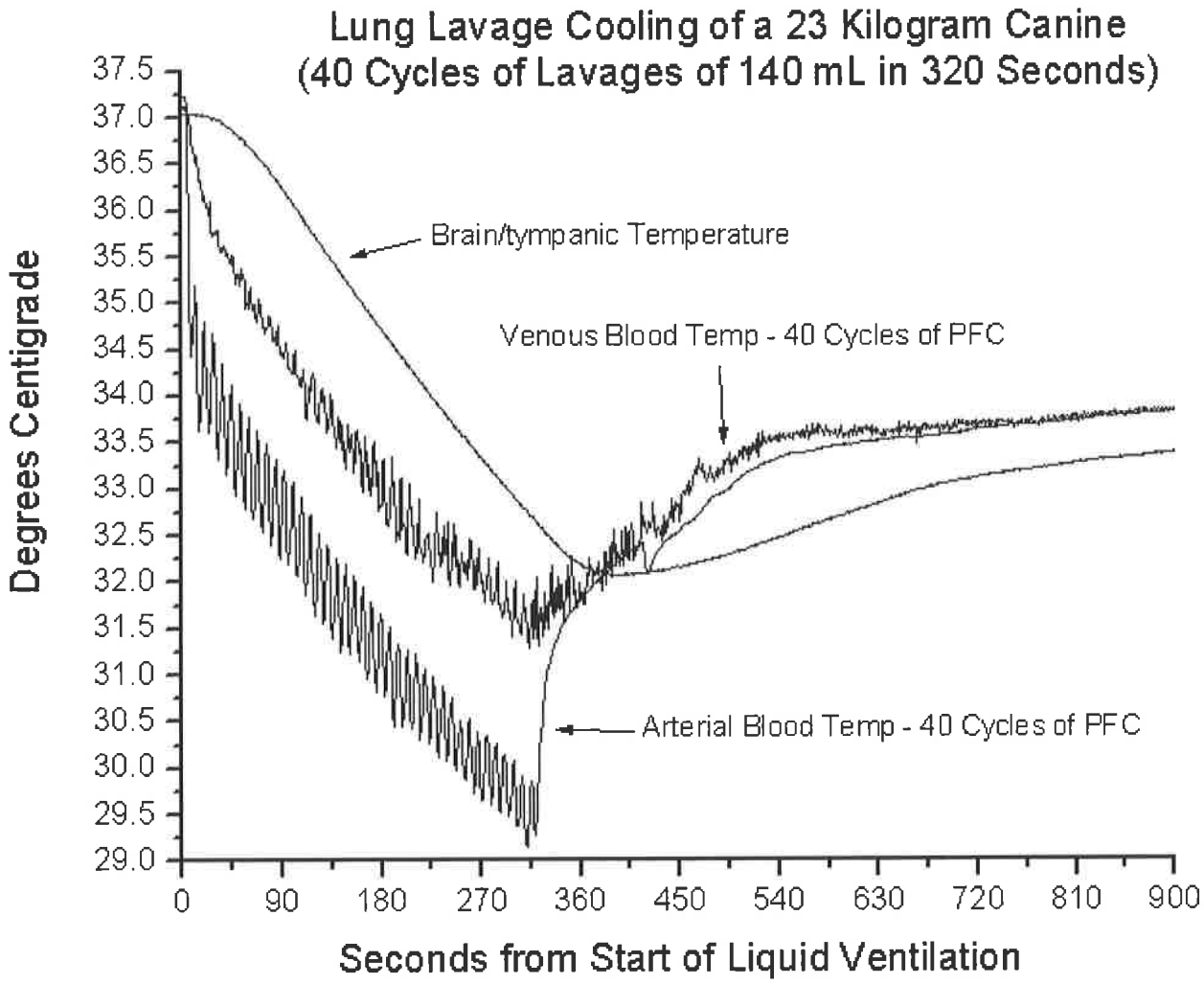

Figure: Animal Lavaged with Device in Patent, 2008. In the Figure above temperatures in the venous system, arterial system, and brain are recorded. Lung lavage is given for 5.3 minutes. Brain temperature does not begin to drop rapidly measurably until 45 seconds, which reflects a circulation delay, as the cooling of the arterial blood is seen to be immediate. However, the overshoot effect occurs after the procedure is stopped, and the minimal brain temperature does not occur until almost 6.75 minutes (a minute and a half after the procedure has stopped), and it is 32.2 °C, which is -4.8 drop from a beginning temperature of 37 °C (98.6 F). Over the next hour the brain will warm to 33.7 °C (-3.3 °C), as the cold core of the body equilibrates with the warmer exterior (about 20% of the total thermal "mass"). In general, continuous lavage of about 6 or 7 minutes is required to guarantee a -4 °C drop at 5 minutes, as well as a permanent brain temperature drop of -4 C even after body and brain equilibration, one hour after liquid ventilation cooling has been stopped. To cool the body and brain permanently by -4 °C requires an early and instantaneous brain cooling of about -5.5 °C, which later develops into a permanent "whole body" drop of -4 °C after equilibration of the cold thermal core with the warmer (but less well perfused) periphery. Thus, the brain is rapidly cooled by -5.5 °C to retain -4 °C.

REVIEW OF LITERATURE

A review of the literature for the USDA confirmed that no other dog models of liquid ventilation cooling are being used in the world.

The liquid ventilation protocol was reviewed with a Medline search and a Google Book Search. Only combined use of the search terms "liquid ventilation" and "hypothermia" returned any papers which were relevant to our live animal liquid ventilation hypothermia protocol. This search returned 22 studies, of which our 2001 study in dogs (PMID 1179148) was the 17th. The remaining 5 studies were older, and had already been noted by us.

No studies have been done with liquid ventilation in dogs to this date, save for ours. It appears that the dog model is a difficult one in terms of having the animals available for study (a dog colony), even though canines it has a number of features making it essential for our own work, including detailed health inspection during recovery of the animal for the next week after liquid lavage cooling. This would be possible in pigs, but these are the only common experiental animal large enough to listen to breath-sounds and assess breathing. However, pigs are far more difficult to manage in extended recovery, and to our knowledge, no group has allowed swine to live for longer than one hour past the procedure.

Staffey and Kerber, (2008) in the swine arrest model (PMID 18406036), allowed resuscitated animals to live for only 1 hour after resuscitation. No pig was allowed to regain consciousness. Staffey notably compared his technique (which was total liquid ventilation using a Harvard pressure ventilator and pre-oxygenated PFC) to cooling by infusion of cold IV saline. He found that oxygenation was better with liquid ventilation than with saline, and his resuscitation was more difficult. He attributed this to a rise in the pressure in the right heart with the significant bolus of saline needed for this degree of cooling (about 4C from body temperature.). He also noted that after 1 hour his swine ventilated with PFCs began to exhibit progressive hypoxia which could not be reversed. He found severe inflammation and edema after an hour on autopsy. He attributed this to large amounts of pulmonary intravascular macrophages which are seen in pigs but not rabbits (which tolerated the procedure better). If this is correct, swine will never been used as liquid ventilation survival model.

Kerber's group paper on this procedure in swine (2009) PMID 19249149 reveals some details: He chilled his PFC to -15 C (far below freezing) but did not speculate as to the damage this might do (we use +1 C). He was able to cool pulmonary blood to 33.8 C in 4 minutes, but this is slower than our rate (femoral arterial blood temp at 29 C at 4 minutes, starting at 36 C). We have found that arterial blood is not a good marker for ultimate brain temperature, since the blood system has only a small fraction of the heat capacity of the system that includes the brain. For example we are required to cool to 27 C arterial temperature at 7 minutes in order to equilibrate to 32 C (-4 C down) in one hour. Thus, the arterial blood cools more than 3 times the amount that is the final brain and body equilibration temperature with very rapid cooling by this method.

However, Kerber used Fluorinert FC-77 in this studies, which we have found far more inductive to pulmonary airway spasm than FC-75 (for unclear reasons, since it is sold as ostensibly a purer form of this chemical). Also, both of these chemicals are far more damaging to the lungs than the two PFCs that we use, FC-84 and FC-40. It may be that the failure of Kerber's swine model due to inflammation is partly due to his poor choice of PCF medium. Also, I this paper the lungs were filled passively to 40 mL/kg with cold -12 to 15 C, fluorocarbon since there was no provision for continued cooing of PFC once the experiment had started. This is a great deal of fluorocarbon, and it was simply allowed to remain in place during chest comparession during cardiac arrest, then drain normally during exhalation when gas ventilation was restarted. Thus, this model is not true total liquid ventilation, but a hybrid of TLV followed by slow drainage of the fluid from the lungs. Moveover, it did not use the amount of PFC we have found is necessary to cool the body permanently by -4 C (about 300 mL/kg) and this is reflected by the fact that this group did not

The longest survival in these papers is in a medium size animal, the rabbit model which is the most common for hypothermia by lavage. The most recent paper is on Ultrafast Whole Body Cooling with Total Liquid Ventilation, performed in rabbits. The group is that of Chenoune and Tisser (2011) at INSERM at Creteil, France. It is reported in Circulation, a prestigious journal. Again the method was TLV—no group has yet used our method of lavage with gas ventilation to cool (although it was used in pigs to warm in 2000). TLV, since there is no gas requires pre-oxygenated cold PFC to perform.

In the Chenoune/Tisser paper (PMID 21810660) they specify that they used only "Fluorinert 3M" which is not sufficient, as 3M makes many varieties of Fluorinert. Chenouune et al. induced cardiac arrest with as shock in rabbits, then resuscitated them after 5 or 10 minutes of arrest. Hypothermia was then accomplished with tidal volume ventilation at 6 breaths per minute (comparable to our lavage rate) at a volume of 7-10 mL/kg body weight (this is a little more than our standard of 6.5 mL/kg body weight in dogs). Acheivement of target hypothermia of 32 C required 10 to 20 minutes, which is about $1/3^{rd}$ of our rate at the same minute-volumes of liquid, suggesting that the heat transfer efficiency in this experiment was only 1/3rd of ours.

However the rabbits were allowed to survive for 7 days. There was an overall 70% survival, but no group was subjected only to TVL, so no direct comparison with our studies is possible in that regard. Necropsy showed apparently little lung damage, and survival of the animals for 7 days is also demonstrative of not a large amount of lung damage (however, the rabbits were kept in oxygenated cages post-resusciation, which is something we rarely do with dogs). Nevertheless, it is difficult to compare studies since this group did not accurately report their FPC perfluorocarbon well enough to be able to be able to repeat their studies. They are convinced that volume of ventilation fluid is the key to their rapid cooling, citing a paper on PFC lung lavage of rabbits done by Yang and Shaffer in 2005, in which cooling by -4 C requires 1 hour.

A number of review of therapeutic hypothermia in humans were included. None are done with liquid lavage. However, all make the point that rapidity of hypothermia induced by

any method, is critical, with every hour lost causing detectable increase in mortality, in human trials using external cooling.

PROJECT 2: Dog Vivarium and Model Development, Breeding Program, and Community Outreach.

We continue to provide upgrades which have caused our USDA inspect to use our kennel as a model for other kennels he works with. These include behavioral modulation programs for our dogs, which results in a nearly noise-free colony, instead of continuously barking animals.

We have one of the few working colonies of research dogs left in the country. Other programs which are forced to rely in liquid ventilation models in rabbits, rodents or non-survival pigs. We believe that our model will only be successful elsewhere with sheep, if at all, but sheep are extremely difficult to monitor for brain temperature and for clinical distress in respiratory experiments (such as wheezing in lung experiments—a prime symptom of damage for us). We believe that our colony provides a unique one in which rapid induction of hypothermia by this method will be developed, or else not at all.

Steven B. Harris, MD
Prepared for Critical Care Research

Brief Annual Report for Critical Care Research for

2011

Notes Prepared For Saul Kent and Bill Faloon
by Steve Harris, Sandra Russell, Joan O'Farrell.

PROGRESS IN CALENDER YEAR 2011

Critical Care Research made progress in 2011, attempting to explore our goal of brain cooling at -1 C/min with a patentable device, without major lung damage. A second version of the device was finished in 2010 and used by us into 2011. The new machine was found to perform excellently, leading to uniform results.

A setback occurred relative to obtaining the correct fluorocarbons for lung lavage, as 3M Corporation discontinued a primary working fluorocarbon, FC-84, which had been a part of our standard mixture FC-40/FC-84. The search for a new fluorocarbon to replace FC-84 in our mixture began last year and has terminated in a suitable replacement in 2011. The replacement fluid Novec ™ 7200 DL (3M Corp) was found unsuitable when used alone due to its high vapor pressure, but following principles we had learned in management of FC-84 we were able to use the fluid in combination with FC-40. Unusual and useful features of this mixture were identified.

The dog continues to be the correct model for lung lavage, since pig lungs have been found by others to react strongly to fluorocarbon even an hour after lavage, and so pig models have not been used as survival models. By contrast, rabbits are too small to assess lung damage during life.

This report is the first to report on the new class of hydroflurocarbon liquids to be used in lung lavage, specifically the liquid hydrofluorcarbon Novec 7200 DL.

Chief Projects.

[1] Test of liquid lavage cooling efficiency by direct methods, with high cooling rates. Tests of pure fluid lavage with Novec 7200 DL and mixture of 7200 DL with FC-40.

[2] Continued experimental dog vivarium management.

SUMMARY PROGRESS

PROJECT [1] Test of Liquid Lavage Cooling Efficiency by direct methods, with high cooling rates. In previous experiments we have found that cold perfluorocarbon

lung lavage in dogs applied in a non-injurious manner consistently delivers at least 0.1 C/min cooling rate to the brain early in cooling (i.e., with at least 30 degrees °C temperature difference between cooling fluid and brain temperature), and that the maximal cooling rate may be as high as 0.5 °C/min (see Harris, S.B. Darwin, M.G., Russell, S.R., O'Farrell, Fletcher, Mike, and Wowk, Brian, Rapid (0.5°C/min) minimally invasive induction of hypothermia using cold perfluorochemical lung lavage in dogs. *RESUSCITATION* 50: 189-204, 2001. PMID: 11719148). This cooling rate was achieved with a type of lung lavage which was a-synchronous with breathing, due to the requirement for gas ventilation at least once every 15 seconds, and the difficulty in complete delivering and removing a large bolus of cold perfluorocarbon in this time. One the other hand, small boluses of fluorocarbon were found to transfer heat less efficiently in our model. For example, the maximal cooling rate achieved in this paper was achieved at a perfluorcarbon lavage rate of 31 mL/kg/min, but this required a lavage time of 37 seconds. The lavage volume for this maximum was 20 mL/kg, but when this was dropped to 9 mL/kg (allowing a lavage time of 16 seconds) the heat transfer efficiency dropped from 60% to 40%, and consequently cooling rates dropped by 30%, even though the lavage rate had increased slightly to 36 mL/kg/min. At the time, we suspected that we had reached a limit, which was due to diminishing heat transfer efficiency, and mechanical efficiency of removal, with small lavage volumes.

In subsequent work, however (detailed in our previous reports) we found that the drop in heat transfer efficiency was avoidable if perfluorocarbon infusion was synchronized with oxygen gas insufflation (best provided by hand bag-valve unit), so that cold perfluorocarbon could always be provided into lungs being expanded by insufflation of pure oxygen gas. We were able to show heat transfer efficiencies as high as 50%, even with lavage volumes as low as 3 mL/kg, with this technique. However, limitations in our equipment (primarily in suction efficiency) still prevented us from conducting lavage faster than 20 mL/kg/min at these small lavage volumes. From theoretical considerations, we understood that we would need total lavage rates of about 60 mL/kg/min to achieve brain cooling rates in the neighborhood of > 1 C/min.

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With the superior suction capabilities of this device, combined with its ability to provide a constant supply of perfluorocarbon cooled to 1 °C (from a reservoir containing only 7 liters of fluid) we performed a series of lung-lavage dog experiments in 2010, using a second copy of this device, which had been improved by Platt to provide for ease in recovering fluorocarbon.

Experiments with the device are possible with collection of the fluid from each lung lavage, which is collected, measured in volume in a separate insulated container, and then analyzed in temperature. This data gives us data for heat transfer that can be estimated

directly from the fluid itself, instead of having to infer it from the dog's estimated heat capacity and degree of cooling. With this device we can calculate the heat removed directly and accurately. This could not be done in measuring fluid temperature during suction (as we discovered) because the temperature and volumes change too fast for a good integration.

A new series of experiments shows that heat transfer to the dog with lavages of 6 ml/kg and 6.5 mL/kg every 7.5 seconds (the maximum that was compatible with little lung damage) gave a heat transfer efficiency of almost exactly 60%.

With the new device we found that 7 minutes of application of 52 mL/kg/min lung-lavage with ice-cold perfluorcarbon (1 °C) results in almost -4 °C drop in brain temperature in the first 5 minutes, and that application of lavage for more than 7 minutes results in a permanent body core temperature of -4 °C (the temporary drop in brain temperature at 10 minutes is almost -6 °C, but this soon equilibrates). The amount of total fluid necessary to cool the entire animal by -4 °C was a consistent 340 mL/kg, or 85 mL/kg/°C. This amount of fluid now sets the standard of ice cold fluorocarbon which is necessary to cycle through the lungs in order to achieve classical "mild hypothermia" for resuscitation. We believe it will also be the amount of fluid necessary per degree of permanent brain cooling in humans.

This cooling corresponds to a final heat capacity of the animal of 0.7 kcal/kg/°C (the same as in humans) with a heat extraction efficiency of 60%. Direct calculation has given us an average extraction efficiency of 62%, making the heat capacity of our average animal slightly higher.

Fluorocarbon replacement in 2011.

In 2010 3M Corporation notified its users that FC-84, the high vapor pressure fluorocarbon used in for electronic circuit board cleaning, would be phased out due to low demand. This liquid had formed 2/3rds of our liquid ventilation mixture (the remainder being FC-40, a more viscous liquid which has a lower vapor pressure). We had found that the superiorly low viscosity of FC-84 made it very useful in maintaining rapid liquid ventilation, even though it was not suitable for use alone (due to rapid infiltration of alveolar interspaces and subsequent emphysema and obstruction of small airways due to lung parenchymal inflation).

Now, 3M was offering no perfluorocarbons in the FC class which had the performance characteristics of FC-84. From previous experiments we knew that FC-40 could be used alone, but that the extra pressures needed to infuse and remove it would result in either lung damage from pressure, or in the need to cut pressures to the point that cooling rates would suffer about about 50% due to slow flows.

The suggested replacement for electronic cleaning uses by 3M included a fluid in the "Novec" class called 7200. This is mostly ethyl nonafluoroisobutyl ether and its isobutyl analogue.

C2H5-0-CF2-CF2-CF2-CF3

C2H5-0-CF2-CF(CF3)2

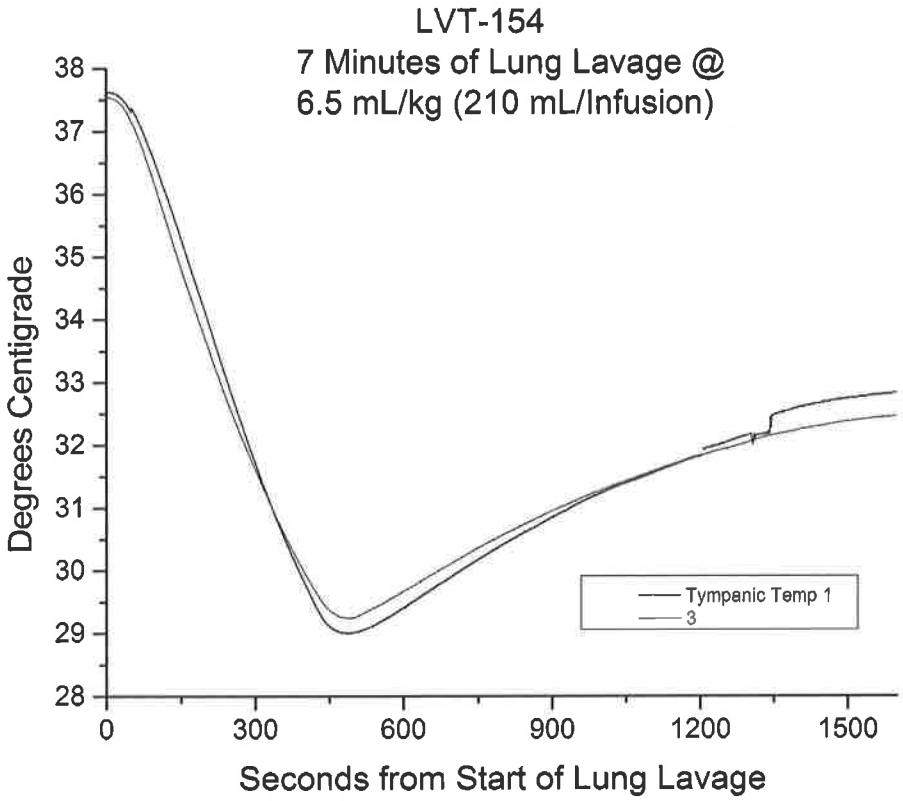
The hydrogens in the ethyl group make this molecule somewhat polar, and it is a chemically a HYDROfluorocarbon rather than a perfluorocarbon. Its viscosity is lower than FC-84 and its vapor pressure even higher, and it is noticeably more hydrophilic than FC-84, with the difference in skin-wetting apparent (the molecule also has a slight odor, which is not apparent with any perfluorocarbon). It is also considerably less dense with a specific gravity of 1.43 rather than 1.70-1.73 typical of Fluorinert FC-84 (which is perfluoroheptane, C7F16). The vapor pressure of Novatec 7200 at 25 C was 109 mmHg, considerably higher than FC-84 at 79 mmHg.

We attempted liquid ventilation cooling of a dog with pure Novatec 7200, which we obtained in pure double distilled form (Novatec 7200 DL), and found that the product is capable of extremely rapid cooling, due to its low viscosity and great rate of suction. However, the animal was almost immediately in trouble due to residual emphysema within a few hours after cooling, and although he survived the next night, he was not able to stand and was in distress despite supplement oxygen. Shortly thereafter he died, the first fatality we have had with liquid ventilation in more than two years.

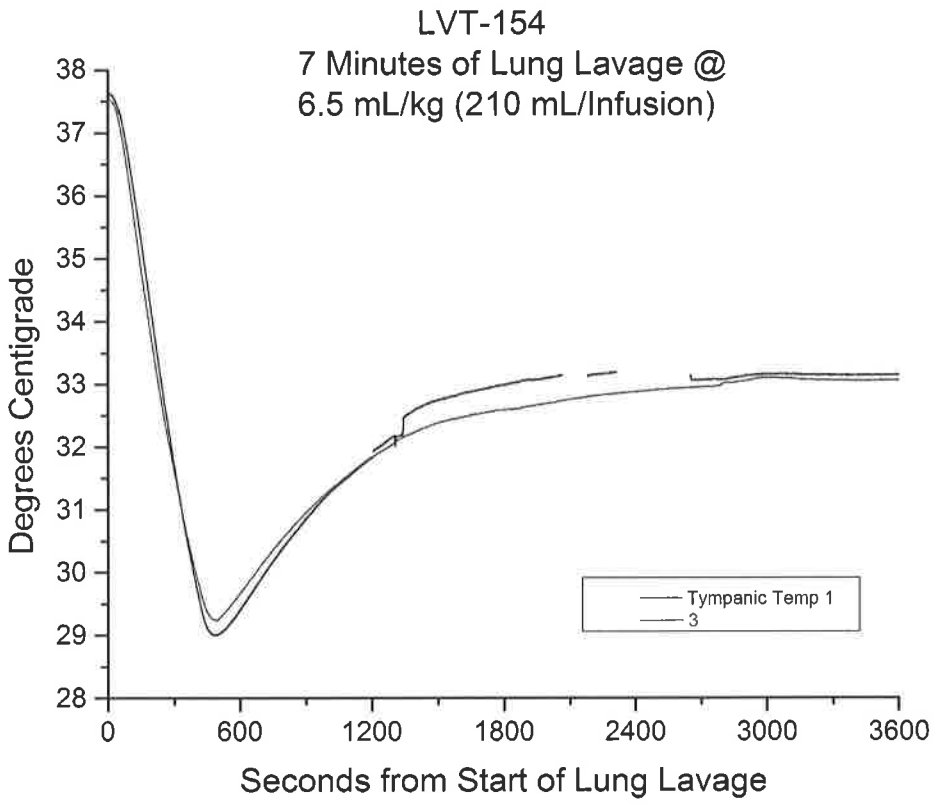
Autopsy showed lungs with bilateral massive emphysema and some bleeding.

Our next experiment required that we test a mixture of FC-40 and Novatec 7200 at a mixture which would provide what we had previously found to be a reasonably safe vapor pressure of 60 mmHg (the calculated pressure of our 2:1 FC-84/FC-40 mix). We mixed FC-40 (which has a vapor pressure of 10 mmHg, or tor) with an equal volume of FC-7200 to obtain a calculated vapor pressure of 65 tor. The density of this mixture was 1.66, less than our previous mixes.

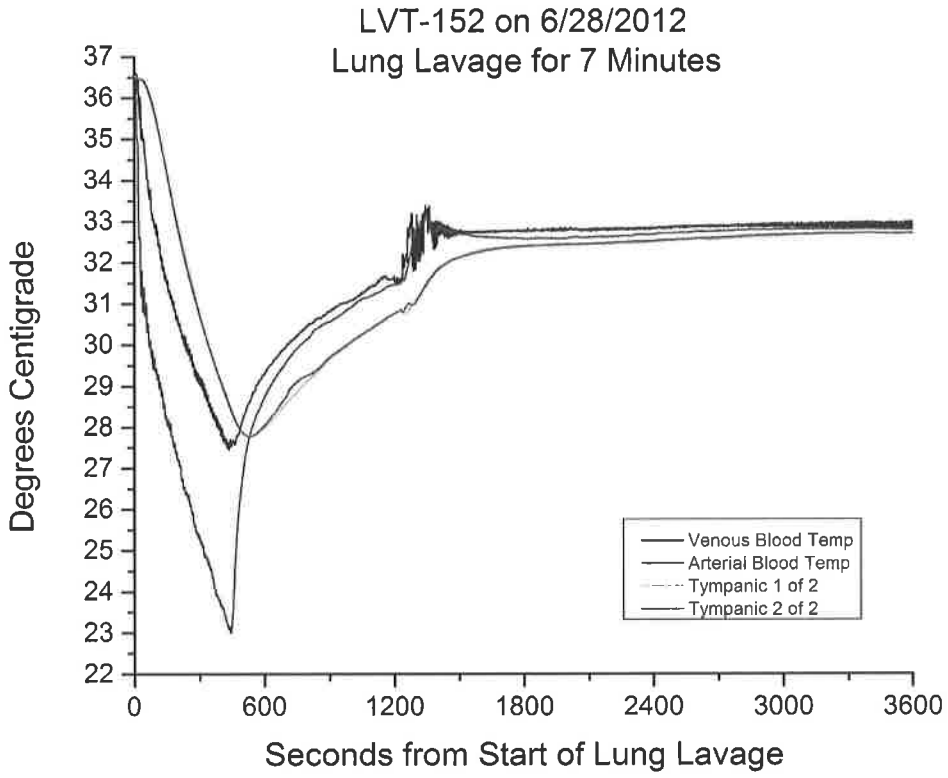
We have now used this 50:50 mix of perfluorocarbon and Novatec 7200 in three dogs and all have survived with only minimal expiratory abnormal breath sounds the next day. However, this mixture seems to cause slightly more lung problems than the previous mixture, in as much as the abnormal sounds last a few days longer. We have a stock of FC-84 (some of the last available, as production has stopped for some time), and expect that it will take time to compare the two fluids. However, the research can apparently continue with this new working fluid in a different class of chemical.



The cooling capacity of FC-40/7200 mix (shown above) is superior to that of FC-84/FC-40, probably due to its ease of recovery in suction, allowing us to use 6.5 mL/kg infusions without problem.



The graph above shows tympanic membrane temperatures after 7 minutes of lavage, showing that the animal's total body temperature has been reduced 4.5 C in this time. The minimal temperature reached was 29 C, which is lower than we have seen in a seven minute experiment. Calculations of efficiency of lavage cooling show heat transfer efficiencies slightly better than the old mix, at 65%.



In the experiment above with the new mix, the dog's venous and arterial systems were monitored with thermocouples (red is arterial temp, blue is central venous). These graphs show that in less than 8 minutes, arterial temperature drops to 23 C, which is more than 13.5 C lower than initial temperature. Maximal rate of change of brain temperature in these experiments (see tympanic temperature curves) exceeded 1.1 C/minute. This dog survived without incident.

These experiments are the first we are aware of, in which the new class of hydroflourocarbons have been used in liquid ventilation of any mammal. This class of chemicals has usually been thought to be too reactive to lung surfactant, and lung water to be useful. However, we find that when combined with a standard high viscosity perflourocarbon like FC-40, the unwanted properties of 7200 can be ameliorated and its useful properties (relatively low density and low viscosity) can be utilized to accelerate the fluid transfer that is needed for heat transfer.

We are the first to show that hydrofluorocarbons can be compatible with normal lung tissue. This may open this entire class of substances to future experimentation in lung lavage work, from treating lung bleeding to smoke inhalation.

PROJECT 2: Dog Vivarium and Model Development, Breeding Program, and Community Outreach.

We continue to provide upgrades which have caused our USDA inspect to use our kennel as a model for other kennels he works with. These include behavioral modulation programs for our dogs, which results in a nearly noise-free colony, instead of continuously barking animals.

We have one of the few working colonies of research dogs left in the country. Other programs which are forced to rely in liquid ventilation models in rabbits, rodents or non-survival pigs. We believe that our model will only be successful elsewhere with sheep, if at all, but sheep are extremely difficult to monitor for brain temperature and for clinical distress in respiratory experiments (such as wheezing in lung experiments—a prime symptom of damage for us). We believe that our colony provides a unique one in which rapid induction of hypothermia by this method will be developed, or else not at all.

Steven B. Harris, MD
Prepared for Critical Care Research

Preliminary Survey of Working Fluids For Mild Hypothermia Induction Via Cold Perfluorochemical (PFC) Lung Lavage

Steven B. Harris; Sandra R. Russell; Joan M. O'Farrell; Brian Hobart; Lu Ho Yu
Critical Care Research, Inc. (Rancho Cucamonga, CA)

Introduction: Post resuscitation mild hypothermia (-4°C) is the first modality to demonstrate a clear therapeutic effect on anoxic brain damage, in randomized human trials (**NEJM** Feb 21, 2002). Cooling was accomplished in ~ 4 hours by air advection in these trials, but animal studies suggest that cooling in the first 15 min after resuscitation would be far more efficacious (**Crit. Care Med.** 21, 1348, 1993). Hypothermic PFC lung lavage (HLL) is the only method known which might be able to cool this rapidly in a field emergency. HLL cools brain/body core in dogs rapidly, allowing mild hypothermia induction in < 20 min. (**RESUSCITATION** 50:187,2001). This study of possible PFC working fluids for HLL was undertaken because the only PFC presently being used experimentally for human partial liquid ventilation (perfluorobromo-octane/Perflubron) is not usable at temperatures below about 15°C, due to viscosity and freezing problems. Commercial PFCs produced for non-medical uses need to be screened and identified for further development in the HLL application.

Hypothesis: Various available fluid PFCs may differ in heat-transfer efficiency (Ef) and clinical outcome in HLL.

Methods: Subjects were 11 anesthetized supine adult dogs (25±1.2 kg), ventilated by 100% O2 by bag/valve. Pre-anesthesia was ketamine/valium/pentobarbital, followed by enflorane gas. Pentobarbital was used during HLL.

Lavage fluids were chosen from available commercial 7-carbon to 14-carbon PFCs. In rank from least to most viscous, and also highest to lowest vapor pressure, these were:

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PROCEDURE

Endotracheal catheters instilled 10 ml/kg lavages of cold (1 to 5°C) PFC in ~12 secs, followed by ~ 25 sec PFC suction. A specially built device was employed for lavage, which uses a portable ice-containing heat exchanger and a computer-driven return-volume-sensitive suction shut-off. Cooling in these circumstances requires about 30 lavages of 10 mL/kg = a total of 300 mL/kg lavage.

Concurrent bag-valve ventilation with 100% O_2 provided all gas exchange, and no oxygenator or soda lime granules in the circuit were used. Dogs were given an extra dose of barbiturate prior to the procedure (during which they were off gas anesthesia).

Dogs were lavaged for 18 min, and tympanic temp (T) measured at 20 min and after T equilibration (40 min). Dogs #1-4 were designated low-viscosity PFC **Group LV**. Dogs #5-11 getting higher viscosity PFCs were designated **Group HV**. Dogs were euthanized and fixative-perfused for lung examination, at 20-24 h post-procedure. Lungs and heart were removed en-block for inspection and densitometry.

Results (±S.E.M.): LV dogs (n=4) received 30±2.7 lavages over 18 min, and cooled (deltaT) by -5.8 ±0.8°C (20 min), and -4.5±0.2°C (40 mins). Mean thermal transfer efficiency (Ef) using Eq 1 was 71±7%. HV dogs (n=7) received 28±2.2 lavages (NS), giving deltaT (40 min) -3.5±0.3 (p=0.08) and Ef 59±6% (NS).

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The pO2 A-a gradient widened in lavage ($p < .001$) and widened further by 24 h post procedure in both groups, but without difference between groups.

All dogs exhibited an asthma-like syndrome the day following lavage. The clinical spectrum ranged from FC-77 worst effects (prostration and gross wheezing), to FC-40 best (normal behavior; nearly quiet lung-sounds). One dog reported here and one other dog in our previous series has had severe asthma after FC-77; this is surprising inasmuch as FC-77 is reported by 3M to be a purer version of FC-75, which has only moderate effects. The high vapor pressure FC-84 exhibited a surprising amount of clinical asthma and obstruction at 24 hours, considering its high clearance by that time.

During ventilation the viscosity differences between fluids made themselves evident in higher pressures required for ventilation with the heavier fluids, and also in the higher incidence of bubbling through the PFC fluid column in the E.T. during O_2 inflation, which was noted with the lighter PFCs only.

At necropsy, HV's PFCs were highly retained by inspection (consolidation), X-ray, and lung densitometry (Density for n=14 lungs, dogs #5-11 = 1.5±.04 g/mL). Dependent areas still containing PFC had a liver-like appearance at necropsy, with hemorrhagic areas of damage, presumably from overpressure, usually confined to non-dependent areas. FC-84 produced a very unusual emphysema-like lung inflation on necropsy, even though mostly cleared by that time on X-ray and densitometry (n=4 lungs D=0.48±.028 g/mL, $p < .001$).

Conclusions:

[1] Mild hypothermia induction requires total lung lavage by HLL of around 300 mL/kg of ice-cold PFC. For at least the first 20 minutes of HLL at 10 mL/kg lavage volume, adequate gas exchange can be accomplished solely by the gas component of ventilation.

[2] For reasons of coordination of lavage with gas ventilation, it is found to be more convenient to carry out gas ventilation with a bag valve device and 100% O2. These conditions are similar to advanced CPR.

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[6] FC-40 is the present best candidate PFC for further development in this model, offering the best clinical picture post lavage. FC-77 was specially toxic in our dog model, for reasons not understood. The more expensive and more highly refined FC-43 appeared to offer no advantages over FC-40. The higher retention of FC-40/43 at 24 hours with respect to lighter PFCs is not a disadvantage, and disappears with time (other dogs not included in this study have eventually demonstrated complete clearing of FC-40 from the lungs).

A Portable Device For Mild Hypothermia Induction Via Cold Perfluorochemical (PFC) Lung Lavage

Steven B. Harris; Sandra R. Russell; Joan M. O'Farrell; Brian Hobart; Lu Ho Yu
Critical Care Research, Inc. (Rancho Cucamonga, CA)

Introduction: Cold PFC lung lavage is capable of cooling brain and body core in dogs at 0.5°C/min. (**RESUSCITATION** 50/2, 187-203, 2001). The technique may allow rapid induction of mild (-4°C) post-resuscitation hypothermia.
Hypothesis: A portable lavage cooler design may use an ice heat-exchange (HEX) module with PFC recirculation.
Methods: A prototype 23 kg device using 1.4 - 2.2 kg of ice and 2.5 liters PFC was constructed. Subjects were anesthetized paralyzed supine adult dogs (17-22 kg), mechanically ventilated (100% O2, 30 breaths/min, tidal volume 15 ml/kg, pressure limit 30 cm H2O). Lavage employed endotracheal catheters to instill 10 ml/kg lavages of cooled (15 ±1.3°C) PFC (FC-75) into the lungs over ~10 secs, followed by PFC suction (~ 40 sec). Gas ventilation provided oxygenation and some CO2 removal; soda lime in the PFC circuit also removed CO2 and H2O. Four dogs were lavaged for 30 mins. Dogs #1-3 received PFC infusion+suction into distal bronchi; Dog #4 received trachial infusion, but bronchial suction. Dogs were euthanized and fixative-perfused at 48 h.
Results (Mean ± S.E.M.): Dogs received a mean total of 373 ± 8.5 ml/kg PFC, and cooled (tympanic temp) by 3.1 ±0.2°C at 32 mins. Mean heat transfer efficiency was 60%. Arterial blood gases @ 0 and 27 min were: pO2 486 ±26 to 368 ±41 torr ; pCO2 39 ±3 to 38 ±2 torr. Dogs #1-3 had increased expiratory breath sounds on exam at 2 days; Dog #4 had normal breath sounds. Necropsy of Dogs #1-3 showed radio-lucent areas of non-hemorrhagic decreased fixation, mostly in caudal/dependent lobes near infusion catheter tips. In Dog #4 such areas were scattered and less sharply demarcated.
Conclusions: A portable lavage cooler requires < 5% of the subject's mass in ice. Pump redesign should allow PFC infusion at present HEX output temp of 3°C. This device demonstrates that even a recirculated-PFC design need not require an artificial PFC oxygenator. Infusion-related minor barotrauma appears decreased by proximal infusion, but distal suction, of PFC.

Keywords: resuscitation, hypothermia, perfluorocarbon

Preliminar Via

Steven B u

INTRODUCTION & HYPOTHESIS

Post resuscitation mild hypothermia (-4°C) is the first modality to demonstrate therapeutic effect on anoxic brain damage, in randomized human trials (NEJM 2002). Cooling was accomplished in ~ 4 hours by air advection in these trials, bu studies suggest that cooling in the first 15 min after resuscitation would be far m efficacious (Crit. Care Med. 21, 1348, 1993). Hypothermic PFC lung lavage (HL only method known which might be able to cool this rapidly in a field emergen cools brain/body core in dogs rapidly, allowing mild hypothermia induction in < (RESUSCITATION 50:187,2001). This study of possible PFC working fluids fo undertaken because the only PFC presently being used experimentally for hum liquid ventilation (perfluorobromo-octane/Perflubron) is not usable at tempera below about 15°C, due to viscosity and freezing problems. Commercial PFCs pr for non-medical uses need to be screened and identified for further developmen HLL application.

HYPOTHESIS: Various available fluid PFCs may differ in heat-transfer efficie and clinical outcome in HLL.

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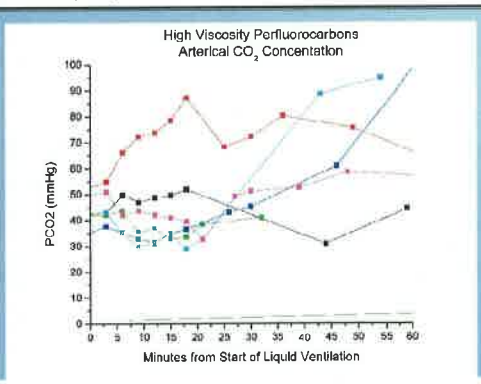

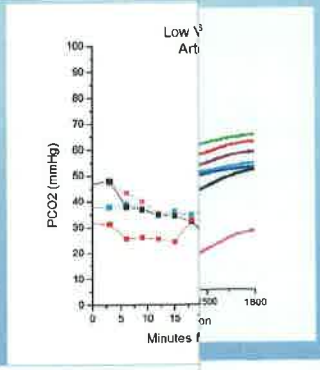

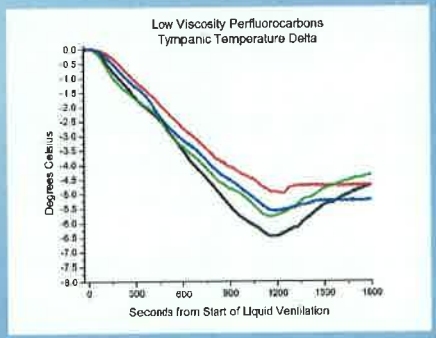

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Steven B. Harris; Sandra R. Russell; Joan M. O'Farrell; Brian Hobart; Lu Ho Yu
Critical Care Research, Inc. (Rancho Cucamonga, CA)

Introduction: Post resuscitation mild hypothermia (-4°C) is the first modality to demonstrate a clear therapeutic effect on anoxic brain damage, in randomized human trials (**NEJM** Feb 21, 2002). Cooling was accomplished in ~ 4 hours by air advection in these trials, but animal studies suggest that cooling in the first 15 min after resuscitation would be far more efficacious (**Crit. Care Med.** 21, 1348, 1993). Hypothermic PFC lung lavage (HLL) is the only method known which might be able to cool this rapidly in a field emergency. HLL cools brain/body core in dogs rapidly, allowing mild hypothermia induction in < 20 min. (**RESUSCITATION** 50:187,2001). This study of possible PFC working fluids for HLL was undertaken because the only PFC presently being used experimentally for human partial liquid ventilation (perfluorobromo-octane/Perflubron) is not usable at temperatures below about 15°C, due to viscosity and freezing problems. Commercial PFCs produced for non-medical uses need to be screened and identified for further development in the HLL application.

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Therapeutic Hypothermia After Resuscitation From Cardiac Arrest.

Brain Damage From Lack of Oxygen Greatly Reduced

A brief summary of progress for this medical treatment (as of 2010), a survey of research in this field conducted in the canine model at Critical Care Research, Inc., and the outlook for continued progress in this field in the future.

Steven B. Harris, M.D.
Critical Care Research, Inc.

Part I. Human Clinical Trials and Recommendations. The human brain can tolerate only so much time without oxygen before permanent damage is done. At normal body temperatures in adults this period is 5 to 10 minutes at maximum, with incidence of severe brain damage or brain death increasing to nearly 100% at the latter interval. Periods of 45 minutes of arrest at 60 °F have been tolerated during neurosurgery, but this requires cardiac bypass cooling prior to stopping blood flow, and similar warming prior to resuscitation and cardiac re-start. These techniques were not applicable to emergency situations in which cardiac arrest occurred at normal body temperatures, due to heart attack or other causes of cardiac arrest.

In the early 1990's post hoc analysis of a series of resuscitation experiments in dogs revealed that accidental anesthesia-related induction of very mild hypothermia (cooling of -7 °F or even less, for a few hours) in the post-resuscitation period, greatly and surprisingly extended the period of cardiac arrest and total loss of brain oxygen which could be previously tolerated at normal body temperatures. The significance of this finding, was that these techniques could be applied in the post-resuscitation period after hospitalization from emergency resuscitation, and no prior preparation or cooling was required.

Safar et al. reported that dogs treated with post-resuscitative hypothermia routinely survived 10 to 12 minutes of circulatory arrest at the close-to-normal canine body temperature of 37.5 °C (99.5 °F), with good neurological scores. Such an insult usually resulted in permanent coma or brain death in animals which were not treated with hypothermia in the resuscitation period. The temperature of 37.5 °C in these studies was chosen as a compromise between normal body temperatures in dogs (about 38 °C) vs. those in humans (about 37 °C). During arrest the animals were not significantly hypothermic, however— as in all these trials, all of the significant cooling happened in the treatment phase after resuscitation.

New human studies soon followed. In 2002, two prospective randomized trials in comatose survivors of out-of-hospital cardiac arrest victims were reported. These trials used patients with return of spontaneous circulation (ROSC) but persistent coma after a witnessed episode of documented ventricular fibrillation (thus insuring that all patients truly experienced no significant brain blood flow). Groups of untreated patients were compared with patients deliberately treated with post-arrest cooling by cold packs or air cooling. The target was about -4 °C of cooling (-7 °C) within 4 hours of ROSC, maintained for 24 hours. The outcomes of these trials were significant, with 55% vs. 39% of patients achieving an independent life in one study, and 49% vs. 26% of patients reaching this goal in the other. [1] [2]

Since these initial studies, research has focused on new and more rapid mechanisms of causing and maintaining hypothermia. Animal studies have suggested that the more rapidly the hypothermia is induced, the better its effect, but the exact parameters remain unknown. Extra-corporal methods are very effective, but are thought to remain too invasive for resuscitation. Peritoneal and pleural lavage with ice-water have been considered. Administration of 30 ml/kg ice-cold saline solution intravenously brings about half of the desired temperature decrease, without causing pulmonary edema. A device which employs intravenous heat exchanger has also become clinically available, which is able to induce the needed -7 °C temperature drop in less than two hours. In January 2003, the Advanced Life Support Task Force of the International Liaison Committee on Resuscitation (ILCOR ALS task force) issued an advisory which formally recommended that mild hypothermia treatment be used in all unconscious adult patients that had suffered ventricular fibrillation, and that the target cooling temperatures should be from 32 °C to 34 °C (89.6 °F - 93.2 °F). The duration of therapy was recommended to be 12 to 24 hours, with normothermia to be restored not actively, but only passively after treatment was discontinued. Methods of cooling, and target rates of cooling could not be addressed, due to lack of hard evidence and experience. It was suggested that in-hospital cooling might be useful for other rhythms and types of in-hospital cardiac arrest. [3]

Part II. Animal Models of Post Resuscitation Hypothermia. Animal models of cardiac arrest, such as rodents and canines, have made it possible to investigate the mechanisms of modulation of post ischemic events in the cerebrum by use of direct brain biopsies and chemical analyses during the post resuscitation period. Such studies have shown that the mechanism of mild hypothermia protection of the brain in the post-ischemia period is nearly as complex as the mechanisms of damage of ischemia itself. A notable feature which stands out from these studies is that the mild degree of cooling in this type of hypothermia does *not* produce a degree of decrease in brain metabolism sufficient to explain the results, so this is not the mechanism by which the intervention proceeds. However, the mild degree of cooling does seem to substantially decrease all of the known post-resuscitation damage-cascades, including free radical release, calcium leakage, toxic neurotransmitter release, other types of inflammation reactions, and apoptosis and programmed cell death. Which of these are more important is unknown, just as the order of their importance remains unknown in normothermic ischemic damage to the brain.

Animal models have also allowed investigation of various methods of experimental cooling which would not presently be possible to use in clinical situations. For example, a study in 1993 by Kuboyama et al. [4] reported that a delay of only 15 minutes from resuscitation to beginning of application of hypothermia, negated the beneficial effects of cooling. Later studies extended this time, but generally found that the sooner cooling was applied, the better for the brain.

Animal models used in investigating mild hypothermia treatment have also allowed the parameter of rate-of-cooling to be more closely controlled, in a way which would not be possible in human clinical trials. In human trials, rate of cooling is required to be set by whatever rate results from safe and clinically acceptable methods. In animal studies, rate-of-cooling may serve as true adjustable target variable, to be set using any method which serves to attain it.

Animal models of cooling using lung lavage with ice cold perfluorocarbon liquids have explored methods of cooling which cool in 1/25th of the 2 hour time needed to induce mild hypothermia (-7 °C) in humans. In animals, this amount of cooling of the brain may be induced in as little as 5 minutes after insertion of an endotracheal tube.

Liquid Breathing as Example of an Experimental Cooling Method. Inhalation of water damages the lungs osmotically when the fluid is either hypo-osmotic (fresh water drowning) or

hyper-osmotic (salt water drowning). However, even iso-osmotic saline causes temporary hypoxia in the lungs after the fluid is removed, via the mechanism of removal of the water soluble surfactant which allows the alveoli to remain open in the presence of great surface-tension forces. Also, saline does not carry enough dissolved oxygen to allow the medical definition of "respiration" (enough oxygen and carbon dioxide exchange to support metabolism).

Perfluorocarbons (PFCs) are molecules in which all of the hydrogen atoms which occupy the non-linking surface positions of a "hydrocarbon" molecule (such as the octane molecule used in gasoline, and many others) are replaced by fluorine atoms. After this modification, the bonded fluorines are difficult to remove, and the PFC molecules become chemically inert. Such molecules are liquids at body temperatures, if they are heavy and complex enough. These PFC liquids do not dissolve in either water or oils, but they are capable of carrying oxygen and carbon dioxide (CO2), which do dissolve in them.

Liquid breathing with perfluorocarbon (PFC) liquids has been investigated since 1965, as a means of allowing gas exchange within the lung by means of a liquid, without removal of the critical surfactant. In the case of fluorocarbon, surfactant is not removed because it is not soluble in the PFC. The lungs can be completely filled with PFC, if it is oxygenated, in a technique called "Total Liquid Ventilation" (TLV). If a liquid ventilator machine adds and removes the PFC from the lungs, and (while outside the lungs) removes the CO2 from it and adds oxygen to it, animals can be ventilated with liquid alone, without bubbles of gas in their lungs. This has been tried on only one human, and then only temporarily. The technique is difficult, the pressures needed close to those which cause lung damage, and the ability to dissolve CO2 and thus remove it from the body is just at the edge of what is needed for dogs and humans.

In 1984 T.H. Shaffer and colleagues investigated PFCs as method of cooling animals. The goal was not mild hypothermia, for this was not then known as a technique. However, there were other reasons to cool and warm animals, and Shaffer was able to show that the TLV cooling technique worked on anesthetized cats. The animals were not allowed to survive the experiment, however, so the long term effects of having the lungs totally full of cooled PFC liquid, were not investigated.

Another related use of PFCs developed later was a technique called Partial Liquid Ventilation (PLV) in which the lungs were filled to 1/3rd of the volume of capacity (about the amount of a normal tidal breath) with PFC, and this was allowed to remain in place while gas ventilation was then carried out "on top" of it. Ventilation was accomplished in the rest of the lung by normal gas ventilation methods. This technique could not be used for heating and cooling (since the amount of PFC was too small to affect body temperature), but it was used to open the dependent parts of the lung and assist with compromised ventilation. It underwent clinical trials in premature infants with a particular PFC perfluorobromooctane (Perflubron™ developed by Alliance Pharmaceutical Corporation). However, this technique was evaluated but not approved by the FDA. In these trials it did not harm the infants but didn't assist them enough to be approved.

Beginning in 1997 and first patented [5] and published [6] in 2001, Critical Care Research, Inc. developed a cooling technique which was functionally a hybrid between TLV and PLV. In this technique, initially called Mixed Mode Liquid Ventilation (MMLV), the lungs are not completely filled with PFC, but instead filled with a volume of PFC liquid similar to PLV, or even a fraction of this volume. This liquid is then periodically infused and removed, passing through a heat exchanger to cool it between cycles. For a canine, volumes of 8 ml per kg were typically used, which would correspond in human adults to volumes of 60 kg x 8 ml/kg = 280 ml = 2 cups of liquid per lavage cycle.

Initially, the PFC was also passed though a gas-exchanger in this technique, to remove CO2 and add oxygen to it. However, as the volume of PFC infused was reduced in experimentation, it was found that this gas replacement was no longer necessary, and that the remainder of the lung was capable of removing CO2 from the animal (and the liquid) and supplying oxygen also, if ventilated with pure oxygen gas. Thus, the gas exchanger for the PFC was not needed, and was eventually discarded.

In 2001, Critical Care Research, Inc. (CCR) published a paper [6] showing that MMLV (later called PFC lung lavage) was able to reduce brain temperature in dogs by a rate as fast as 0.5 °C per minute. Thus, the needed state of mild hypothermia (-4 °C cooling) could be induced in a time less than 10 minutes (allowing for heat transfer delays). This was a factor of 12 faster than the 2 hours required by the intravenous techniques, the fastest cooling technique which had been reported by any method.

Cardiac bypass techniques were known to be able to cool in excess of 1 C per minute, but circulatory bypass involves gaining access to major blood vessels (usually the femoral arteries and veins in the groin) and there is a necessary delay time to initiate bypass after an emergency. It also requires a skilled vascular surgeon. PFC lung lavage, by contrast could be initiated as soon as an endotracheal tube was in place in the patient's "windpipe", and in theory could be done in the field, by paramedics.

Development of Lung Lavage. In the years that followed, the CCR technique was confirmed as PFC lung lavage was used by other groups to warm adult pigs, and to cool rabbits. However, it did not undergo further development in most centers due to lack of NIH enthusiasm, and the perceived difficulty of gaining approval for a new device and a new experimental "drug" (the needed PFC) to accomplish the clinical technique. The PFC needed for the technique, due to constraints in freezing, could not be the Perflubron™ which becomes too thick to use at 15°C (nearly ice-cold PFC, at 2 to 3 °C is needed for best results). Eventually a PFC used in the electronics industry was located which filled the needs of the technique.

Unlike other experimental labs, CCR normally allowed many of its experimental animals (canines) to survive long term, after the procedure. It was found by this protocol that an asthma-like syndrome was produced by high rate lavage with many PFCs, but that this did not become appreciable until 24 hours after lavage-cooling. It was never fatal, but did cause the animals difficulty breathing for as long as week after the procedure. Eventually, as with all PFC procedures, the PFC evaporates and disappears from the lungs, and the animals recover completely

Over the course of the next 8 years, CCR has made an exhaustive study of the necessary parameters of lung lavage to minimize the post-lavage syndrome. These include investigation of 6 different candidate PFC fluids, and exploration of the optimal lavage volume, timing, temperature, and method of delivery and mode of removal. Because PFC is heavy and resists suctioning, the optimal methods to introduce and remove it turned out to require a dedicated apparatus, but once constructed, this apparatus proved easy to use, well within the abilities of paramedics to employ, in CCR's estimation.

At the same time this development was taking place, Lance Becker at Argonne National Laboratories had been working with tiny ice spheres which could be suspended in PFC as a slurry, and delivered to the lungs. The phase change as the ice melts provides an extra degree of cooling power per volume of lavage which PFC alone does not, but on the other hand, the saline

generated in this way also presumably dissolves some lung surfactant. Becker reported initial tests of this system in euthanized animals, but did not end up developing it further (as of this writing). As in many of these systems, since the animal was not allowed to survive, we do not know the long term effects of the procedure.

Part III. Challenge of Becker, et al. to **produce ultra-rapid brain cooling in a large animal.**

In 2007, Dr. Becker and Joshua W. Lampe published a paper [7] in the journal **Expert Review of Medical Devices,** in which they challenged experimenters to design a cooling system which would lower brain temperature by 4°C in the first 5 minutes of application. They wrote:

*"The induction of mild hypothermia, lowering body temperature by 4°C, is gaining acceptance as an acute therapy for the treatment of hypoxia and ischemia following cardiac arrest and many life-threatening injuries. When hypothermia is used following ischemia (as opposed to before ischemia), it needs to be performed rapidly for the greatest benefit, preferably within **5 min**. A simple model reveals that this poses a significant bioengineering challenge as the rate of heat transfer is severely limited, owing to a relatively confined fundamental parameter space. Current methods of cooling include external cooling devices, such as cooling blankets or ice bags, which are simple to use, relatively inexpensive but slow. Internal cooling has the best ability to cool more rapidly but current devices are more invasive, costly and most are still not able to provide cooling within the rapid 5-min interval."*

Lampe and Becker go on to a discussion of theoretical amounts of heat which can be removed by a number of methods, including cardiac bypass, but note that the surgery for this takes at least 1 hour to implement. They conclude:

"Hypothermia of 3-4°C must be achieved within 5 min for maximum protection when required after a period of ischemia. The human body is well designed to prevent heat loss, creating a significant bioengineering challenge in a relatively confined fundamental parameter space. The minimum temperature and the maximum volume of coolant that can be introduced into the body are significant engineering obstacles."

In reading Lampe and Becker's article, CCR realized that Becker and Lampe had seriously miscalculated the amount of heat which would be need to be removed during rapid cooling, due to their inexperience with the decrease in effective heat capacity of the body, which occurs during very rapid cooling of the blood. CCR's previous experiments had found that only a 70% "core" mass (which includes the brain, fortunately) is cooled in the first few minutes of cooling, and that if cooling continues past 5 minutes, the extra heat to be extracted in the 6^{th} and 7^{th} minute will make up for the later "re-equilibration" which takes place within the animal or human as the cold core and brain are re-warmed by the periphery (which does not initially cool as fast as they do). The differential cooling of the thermal core during very fast cooling allows a temporary window in which only part of the body (including the brain) needs to be cooled, with the catch-up period of re-equilibration later, used for compensatory cooling.

Since Lampe and Becker had also assumed that the heat capacity of the body is that of and equivalent amount of water (it is only 70% as large) their estimates of needed heat extraction were off by a factor of 70% of 70% (a factor 2). Also, their estimations of the amount of cold fluorocarbon which could be delivered to the lungs, was incorrect. At CCR it was realized that the challenge set by Lampe and Becker had already nearly been met.

In 2007, CCR had been experimenting with a breadboard prototype lung lavage machine using

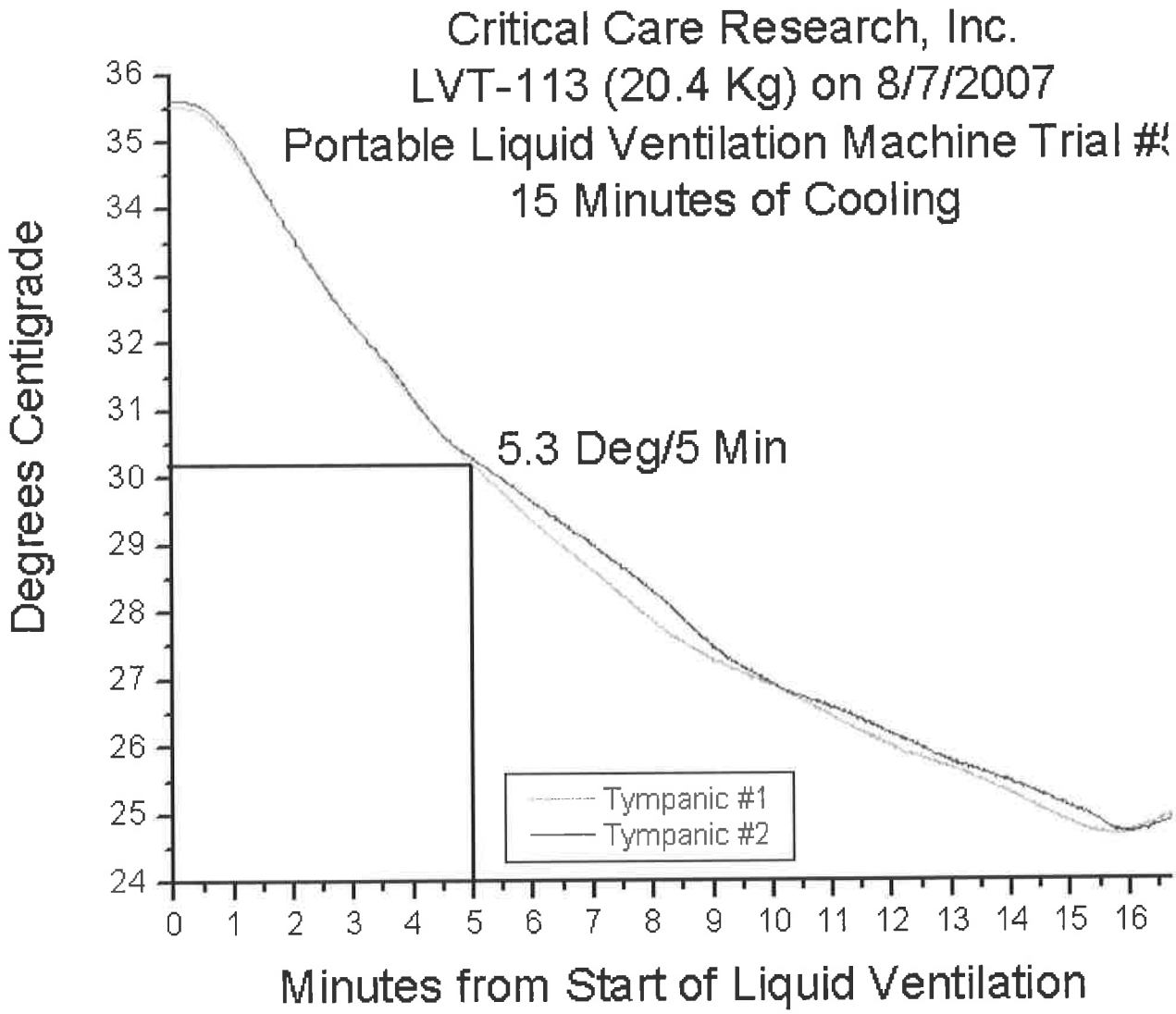
peristaltic pumps (i.e., "roller-type" pumps which squeezed flexible tubing, as in a older traditional heart-lung machines (these machines are now beginning to use centrifugal pumps, which cannot be used for our purposes, due to their incompatibility with air). Although these early peristaltic type pumps were adequate for fluorocarbon suction, they were not able to provide the very high suction rates which were required to return 60 ml/kg/min of PFC, which was estimated would be the minimum to achieve Lampe and Becker's goal of -4 °C in 5 minutes. [Because of initial delays in blood transfer of heat to the brain, this would require maximal cooling rates of > 1 °C/min, probably in the range of 1.2 °C/min].

The major reason for this failure was the failure of these pumps to be able to return not only the 60 ml/kg/min of PFC required, but also the volumes of air from the lungs, which amounted to several times this volume, and which had to be removed before the lungs "deflated" to the point of being able to remove sufficient PFC liquid.

Work on Portable High Capacity Lung Lavage Device. Beginning in 2007 CCR contracted with outside engineering consultants to provide a suitable high capacity (high suction) machine, which would be completely portable (able to run from batteries) and also able to cool the body more than -4 °C, should this be required. In designing this machine, CCR made use of the high-capacity commercial "plate-type" heat exchangers already available on the commercial market for other uses (these are capable of nearly 600 watts/degree gradient of heat transfer between ice-water and PFC). CCR then discarded the bulky peristaltic pumps and replaced them with inexpensive commercial fluid diaphragm pumps, which proved far superior, and able to run on low voltage D.C.

With the superior suction capabilities of this device, combined with its ability to provide a constant supply of perfluorocarbon cooled to 2 °C (from a reservoir containing only 7 liters of fluid) CCR performed a series of 30 lung-lavage dog experiments in 2007 and mid 2008. The first results were encouraging enough to apply for a preliminary patent on the device in September 2007, and a full patent application was filed (Platt, Battiano, Harris) for the device in September, 2008 [8].

A graph from one of the early experiments with this device, after design parameters had been partly adjusted, illustrates performance:



This graph shows that Lampe and Becker's stated goal (which they thought impossible without a direct application of a phase-change substance such as ice to the body) of -4 °C in 5 minutes has been exceeded with the device, which provided -5.3 °C in the first 5 minutes of application . In addition, in this experiment, CCR was able to cool the animal's brain and heart to 25°C (77 °F), in just 16 minutes.

Later experiments with the device show that 6-7 minutes of application of 60 ml/kg/min lung-lavage with ice-cold perfluorcarbon (2 °C) results in at least -4 °C drop in brain temperature in the first 5 minutes, and that application of lavage for more than 6 minutes results in a permanent body core temperature of -4 °C. All of these results are novel, and should be of great importance to the resuscitation community.

In numerous repeatable experiments CCR found that 6 minutes of lung lavage is required to result

in a permanent -4 °C temperature drop, as well as an instantaneous drop of -4 °C in the first 5 minutes of lavage. This is due to the fact that the first minute of lung lavage results in almost no measured brain cooling, due to heat exchange delays while the blood is cooled via the lungs, and the "cold" is transferred to the brain. However, when lavage is stopped, cooling of the brain continues for nearly two minutes, while the blood volume and the lungs (which may be as much as 2.5 °C colder than the brain, or in other words, at 29 °C when the brain is at 32.5 °C) go on to equilibrate with the brain and the central tissues of the animal. Cooling of the core of the animal of more than -4 °C, to as low as -6 °C, is also required so that the rebound heading of the core, which happens in the next 20 minutes, does not succeed in re-warming the core above 33 C (-4 °C of cooling). However, all this can be done in 6 minutes, and the -4 °C which occurs at the end of the 5th minute, is thus "permanent" in the sense that it is not removed by re-equilibration of heat within the body later.

Note that all these problems (and benefits!) of having the core of the animal substantially cooler than the periphery, only happen in extreme rates of cooling, such as are not seen in any other cooling techniques applicable to the field in emergencies. However, they now serve as part of the basic technique at CCR.

The post-lavage asthma syndrome which is exacerbated by wrong choice of PFC, and by use of the wrong techniques during lavage (which result in needless overpressures), has also been overcome by CCR. This syndrome is not known by other groups, because CCR presently has the only large-animal model of lung lavage, where the animals are allowed to survive long enough to develop later lung reactions. [CCR maintains its own canine dog colony, with all animals bred "in house" since 2003).

The present extreme cooling technique causes little or no asthmatic reaction in dogs, and they are clinically comfortable, and survive. Dogs euthanized for examination of the lungs at the time of maximal asthma show no small lung hemorrhages (these are a very sensitive indicator of lung damage, inasmuch as dogs with damage that produces many small hemorrhages will survive anyway).

Construction of a second, smaller, and more efficient prototype of this machine is presently underway.

The following graph shows the performance of this machine in detail, when used for 5.3 minutes to cool a dog's brain by -4.8 °C in 6.75 minutes, slightly less than the best performance seen in the graphs above. However, this dog has been fully instrumented, so that the instantaneous change in centeral venous and arterial blood temperatures (in the aorta and inferior vena cava) can also be seen. Note the excursions in both venous and arterial blood with each of the 40 lavages (one lavage every 8 seconds) in this experiment. Blood temperatures fall as low as 29.2 °C (84.6 °F) in this animal which begins at a temperature of 37 °C (98.6 °F). At the end of the experiment, equilibration of venous and arterial blood can be seen, followed by equilibration of the entire blood pool as a whole with the core of the animal, as represented by the brain temperature (this happens after about 700 seconds or a bit less than 12 minutes, when blood and brain temperatures begin to track in parallel). Note that blood temperature still exceeds brain/core temperature by about 0.4 °C at 900 seconds (15 minutes) because at this time, blood is still carrying heat from the warm periphery of the animal to the cooled core tissues, which include the brain (that is, full body tissue and compartment heat equilibration is not complete).

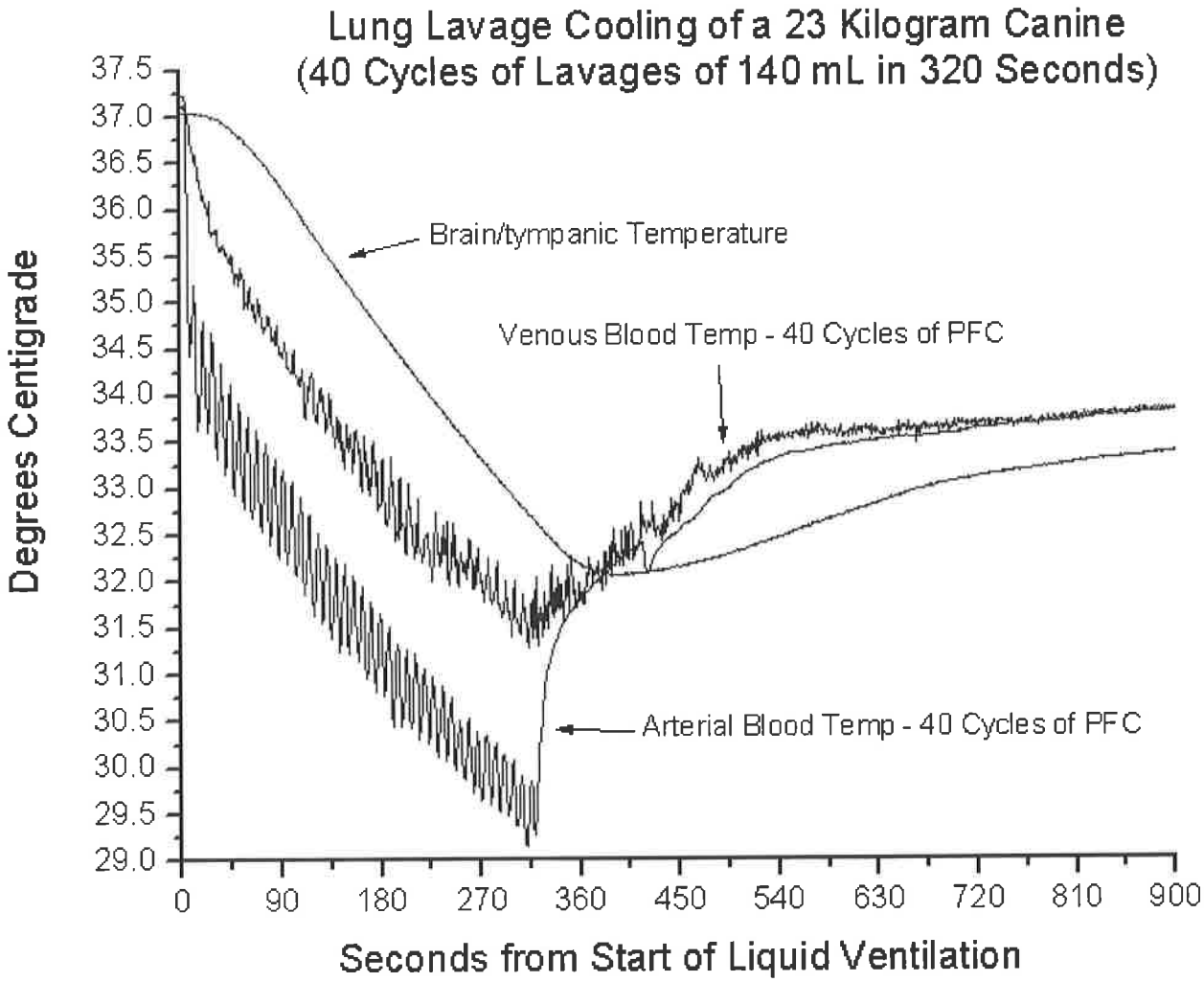


Figure: Animal Lavaged with Device in Patent, 2008. In the Figure above temperatures in the venous system, arterial system, and brain are recorded. Lung lavage is given for 5.3 minutes. Brain temperature does not begin to drop rapidly measurably until 45 seconds, which reflects a circulation delay, as the cooling of the arterial blood is seen to be immediate. However, the overshoot effect occurs after the procedure is stopped, and the minimal brain temperature does not occur until almost 6.75 minutes (a minute and a half after the procedure has stopped), and it is 32.2 °C, which is -4.8 drop from a beginning temperature of 37 °C (98.6 °F). Over the next hour the brain will warm to 33.7 °C (-3.3 °C), as the cold core of the body equilibrates with the warmer exterior (about 20% of the total thermal "mass"). In general, continuous lavage of about 6 minutes is required to guarantee a -4 °C drop at 5 minutes, as well as a permanent brain temperature drop of -4 °C even after body and brain equilibration, one hour after liquid ventilation cooling has been stopped. To cool the body and brain permanently by -4 °C requires an early and instantaneous brain cooling of about -5.5 °C, which later develops into a permanent "whole body" drop of -4 °C

after equilibration of the cold thermal core with the warmer (but less well perfused) periphery. Thus, the brain must be rapidly cooled by -5.5 °C to retain -4 °C.

N.B., in dogs the tympanic temperature is a reliable indicator of brain temperature, but in humans it is not. Dog brain temperature in this experiment is also guaranteed by direct measurement of dog venous blood temperatures, which must (after lung-blood heat transfer stops) closely track brain temperature over equilibration-time scales longer than about 2 minutes. In this model, the temperatures of blood and brain/core would be the same if it were not for a slow heat "leak" into the blood from the yet-to-be-cooled warmer periphery.

Part III. The Future of Post Resuscitation Hypothermia. The technique of post-resuscitation cooling, even with simple ice-bags in the Emergency Department, has been slow to catch on. Problems include logistical difficulties and even lack of advertising to move information (there are no large pharmaceutical companies involved). A 2005 survey of 256 emergency room physicians found that despite official ILCOR endorsement of this technique for Advanced Life Support (ALS) in 2003, only a quarter of these physicians were using the technique on their resuscitated patients.[9] The reasons given were that the technique was difficult or slow, as well as a technically correct but misguided assertion that the ALS guidelines did not incorporate it (these guidelines had not been updated since 2000, at that time, so they had not been able to incorporate cooling recommendations).

Moreover, simple ice-bag techniques as used in emergency departments are ineffective at cooling often. A recent (2009) study reported in **Journal Watch Emergency Medicine** reported that post-resuscitative cooling of 287 patients with ventricular fibrillation arrest, using ice packs, cooling blankets and cooling pads, reached the target temperature of 32 °C – 34 °C in only 65% of patients. [10] Despite this, the group found that treatment increased survival to hospital discharge from 395 to 54% and favorable neurological outcome rate from 15% to 35%. They said wrote: "…it is time for [Emergency Departments] to implement hypothermia protocols for comatose survivors of cardiac arrest."

These and other results have continued to amaze physicians who had believed that the traditional 5 to 10 minutes of warm circulatory arrest time (without chest compression) was the limit, after which brain death was certain. A recent **Wall Street Journal** article quoted one "early-adopter" of the post-resuscitation cooling protocol [11]:

"We've had patients who have been stone-cold out for least 20 minutes—we know that for sure—and they've come back normal or nearly normal," says Michael Mooney, a cardiologist who heads the therapeutic hypothermia program at Minneapolis Heart Institute. An early adopter of the cooling technique, the cardiology practice has treated more than 140 patients since 2006 and says 52% have survived, compared with single digits historically; of those, about 75% have had a "favorable neurologic recovery," including many who report a full return to normal.

The difference between 10 minutes and 20 minutes is particularly important, for this is the average paramedic response time for a large fraction of urban areas in the country. Nor are physicians sure that 20 minutes is the limit for resuscitation, as these studies are still being done with mostly conventional methods of looking, with ice packs and esophageal ice water lavage, which takes at least several hours.

The Future of Lung Lavage. Critical Care Research, Inc. has already solved many of the key problems in inducing very rapid brain mild hypothermia (-4 °C by 5 minutes of lavage, sufficient heat removal for a permanent -4 °C after equilibration, after 6 minutes of lavage), with minimal post procedure lung reaction in the following days. Remaining, however, are the difficult and expensive clinical trials which must occur in humans.

Dogs, which more delicate and slightly larger lungs (for their body size) than do humans, are a good model for lung lavage. Although it may be possible to cool a dog slightly more quickly than a human by this method, at the same time, if the technique is does not damage dog lungs at a given pressure, it probably will not damage human lungs. Initial clinical trials in humans will need to be carried out at a fraction of the maximal rates and pressures which have been achieved in canines.

Lung lavage remains to be tested after resuscitation, in an actual large-animal model of brain ischemia and circulatory arrest.

Other uses for PFC lung lavage: Although repeated PFC lung lavage was developed at CCR for heat exchange purposes, it can also be used with normal body temperature PFC, for purposes of mass-exchange (which closely parallels heat exchange). This technique would remove particles and fluids from lungs which are impossible to remove in any other way. For example, smoke particles may be dislodged by PFC for treatment of smoke inhalation. Also, the toxic fresh-water and sea-water remaining in the lungs after near-drowning will be expected to float on the top of PFC liquid without mixing with it, where it can be recovered from the deep lung to rise and be suctioned off with the upper layer of PFC in an open-circuit application the technique (where PFC delivered to the lungs is not returned after removal). Lungs filled with cardiac failure edema, and possibly with inflammatory exudates from pneumonia or other problems, could also be cleansed by these techniques.

All of these problems remain to be explored, and will benefit from the knowledge gained by CCR from its unique large animal model of rapid PFC lung lavage.

SUMMARY. The advantages of therapeutic cooling for post-resuscitation scenarios have been admitted by all, at this time. The main question remaining is how best to do it, and how rapidly it must be done. Although the question of what the best rate of cooling and what the critical delay is in providing it, cannot be answered without more data, certainly the most conservative answer is that least damage to the brain is likely to be done if the entire cooling "job" can be done in the time-frame that we already know is relatively "safe" for the brain to be without any oxygen, which is about 5 minutes. Thus, the most convincing argument for rapid cooling is made by Lance B. Becker, M.D., a founder and Director of the Emergency Resuscitation Center at the University of Chicago in Chicago and Argonne National Laboratory [12]. If Dr. Becker's argument for the need for rapidity in cooling is accepted, then the method which unquestionably has the best chance to cool the human brain (and body core, since they cannot be separated, due to high connecting blood flow) by -4 °C, in less than 5 minutes, is PFC liquid lung lavage [13]. This method approaches the rates available in cardiopulmonary bypass cooling, but without the need for circulatory bypass or surgery. Only intubation is required.

Other ultra-rapid methods have been considered, but they all require introduction of too much volume into the intravascular space to be practical. An infusion of 30 ml/kg of ice cold saline

appears to be close to the limit of intravascular infusion, and this permanently would cool the body by only
-1.6 °C or so. Following this, other techniques would need to be used. In some cases, these large fluid volumes could not be used at all. The implied argument for something like PFC lung lavage cooling was made by Dr. Becker in discussing alternatives, before he was aware the capability of lung lavage. Here is what he writes [7]:

The induction of mild hypothermia, lowering body temperature by 4 degrees C, is gaining acceptance as an acute therapy for the treatment of hypoxia and ischemia following cardiac arrest and many life-threatening injuries. When hypothermia is used following ischemia (as opposed to before ischemia), it needs to be performed rapidly for the greatest benefit, preferably within 5 min. When we consider the basic heat-transfer problem and define the engineering parameter space, we find that almost 3900 W of cooling are required in order to achieve 4 degrees C cooling within 5 min. A simple model reveals that this poses a significant bioengineering challenge as the rate of heat transfer is severely limited, owing to a relatively confined fundamental parameter space.

Current methods of cooling include external cooling devices, such as cooling blankets or ice bags, which are simple to use, relatively inexpensive but slow. Internal cooling has the best ability to cool more rapidly but current devices are more invasive, costly and most are still not able to provide cooling within the rapid 5-min interval. Cardiopulmonary bypass and recirculating coolants can achieve the cooling rate but are currently extremely invasive and require a highly skilled team to implement. Future therapies may include phase-change coolants, such as microparticulate ice-saline slurries or evaporative cooling technologies specifically designed for human use. With continuing research and investment, methods for rapid cooling can be developed and will translate into saving lives.

In fact, the method which Dr. Becker is asking for, is already available in animals, because for the design "fundamental parameter space" he set, was not the correct one. It remains to test the known canine solution in humans, but because of simply biomechanical equivalencies in heat transfer [14], the odds are extremely good that it will applicable.

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12. For more on Dr. Becker, see http://www.med.upenn.edu/apps/faculty/index.php/g20000140/p8123041

13. Various devices for cooling the head-only have been considered by experimenters, but cephalic blood flow is roughly 1 liter/min, which is far too high to counteract by any conceivable device which cools only the head.

14. The major inequality between humans and dogs is the dog's 30% larger lungs per body mass. The relative differences in brain size per body weight is not a factor, since the brain must be cooled as part of the body's thermal core, which is 70% of the heat capacity of the animals, whether canine or human

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Rapid (0.5°C/min) minimally invasive induction of hypothermia using cold perfluorochemical lung lavage in dogs

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Abstract

Objective: Demonstrate minimally invasive rapid body core and brain cooling in a large animal model. *Design:* Prospective controlled animal trial. *Setting:* Private research laboratory. *Subjects:* Adult dogs, anesthetized, mechanically ventilated. *Interventions:* Cyclic lung lavage with FC-75 perfluorochemical (PFC) was administered through a dual-lumen endotracheal system in the new technique of 'gas/liquid ventilation' (GLV). In Trial-I, lavage volume (V-lav) was 19 ml/kg, infused and withdrawn over a cycle period (tc) of 37 s. (effective lavage rate V'-lav = 31 ml/kg/min.) Five dogs received cold (~4 °C) PFC; two controls received isothermic PFC. In Trial-II, five dogs received GLV at V-lav = 8.8 ml/kg, tc = 16 s, V'-lav = 36 ml/kg/min. *Measurements and main results:* Trial-I tympanic temperature change was -3.7 ± 0.6 °C (SD) at 7.5 min, reaching -7.3 ± 0.6 °C at 18 min. Heat transfer efficiency was 60%. In Trial-II, efficiency fell to 40%, but heat-exchange dead space (VDtherm) remained constant. Lung/blood thermal equilibration half-time was < 8 s. Isothermic GLV caused hypercapnia unless gas ventilation was increased. At necropsy after euthanasia (24 h), modest lung injury was seen. *Conclusions*: GLV cooling times are comparable to those for cardiopulmonary bypass. Heat and CO_2 removal can be independently controlled by changing the mix of lavage and gas ventilation. Due to VDtherm of ~6 ml/kg in dogs, efficient V-lav is > 18 ml/kg. GLV cooling power appears more limited by PFC flows than lavage residence times. Concurrent gas ventilation may mitigate heat-diffusion limitations in liquid breathing, perhaps via bubble-induced turbulence. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Resuscitation; Hypothermia (induced); Brain ischemia; Spinal cord injury; Perfluorocarbons; Fluorocarbons; Respiration (artificial/methods); Dogs; Dead space (respiratory); Lavage

Resumo

Objectivo: Demonstrar a redução rápida da temperatura central e cerebral num modelo de animal de grande porte com um método minimamente invasivo. *Desenho:* estudo controlado e prospectivo em animais. Contexto: laboratório de pesquisas privado. *Sujeitos:* Cães ventilados mecanicamente e anestesiados. Intervenção: lavagem cíclica dos pulmões com FC-75 penfluorado (PFC) através de um tubo traqueal de duplo lumen, recorrendo à nova técnica de ventilação gás/líquido (GLV). No ensaio I, o volume da lavagem (V-lav.) foi de 19 ml/kg, introduzido recuperado em períodos cíclicos de 37 s. Taxa de lavagem efectiva V-lav. = 31 ml/kg/min. A cinco dos quais foi feita PFC a cerca de 4 °C. Dois grupos de controlo receberam PFC isotérmico. No ensaio II cinco dos cães receberam GLV a V-lav. = 8.8 ml/kg, tc = 16s, V'-lav. = 36 ml/kg por minuto. *Medições e resultados principais:* No ensaio I a temperatura no tímpano baixou-3.7 ± 0.6 °C aos 7.5 min e a -7.3 ± 0.6 °C. A eficiência de transferência de calor foi de 60%. No ensaio II, a eficiência reduziu-se a 40% mas os humidificadores, o espaço morto(Vdtherm) permaneceram constantes. O tempo de equilíbrio térmico do conjunto coração/pulmão foi < 8 s. A GLV com normotermia provocou hipercapnia a menos que o volume corrente fosse aumentado. À autópsia, depois de sacrificar os cães, as lesões pulmonares encontradas eram escassas. O tempo de arrefecimento é comparável aos dos 'bypass' cardiopulmonar. A mudança da forma de fazer a lavagem e da composição dos gases permitem controlar de forma independente o calor e a CO_2. Como nos cães a "Vdtherm" é de cerca de 6 ml/kg, a V-lav é > 18 ml/kg. A capacidade de arrefecer da GLV parece ser mais limitada pelo fluxo

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do PFC, do que o momento da lavagem A utilização em simultâneo de ventilação por gás pode dificultar o arrefecimento, provavelmente pela turbulência provocada pelas bolhas de gás.

Palavras chave: Reanimação; Hipotermia (induzida); Isquemia cerebral; Lesão da espinal medula; Perfluorcarbonados; Fluorcarbonados; Cães; Espaço morto (respiratório); Lavagem

1. Introduction

Mild hypothermia ($\Delta T = -2$ to -6°C) during ischemia [1] and reperfusion has been called the gold standard against which neuroprotective strategies must be measured in the research setting [2]. However, despite excellent results in controlled animal models, clinical application of post-insult hypothermia has been problematic, due primarily to the logistics of achieving very rapid systemic cooling after injury [3]. The optimum therapeutic window for the treatment of CNS injury using hypothermia remains unknown, however results from hypothermia treatment of burns and sports injury suggest by analogy that the therapeutic window for all post-injury hypothermic treatment may be narrow. Indeed, it has been reported that in one dog model of cardiac arrest, even a 15 min delay after injury negates most of the considerable CNS-protective effect of post-insult hypothermia induction [4,5]. The utility of mild hypothermia treatment for human CNS injury may therefore require the ability to very rapidly cool the CNS and body core [6].

Several systemic cooling modalities are available. The most rapid and invasive of these is cardiopulmonary bypass (CPB). CPB is limited to cooling rates of approximately 1°C/min, due to RBC aggregation and the danger of gas embolism as chilled gas-saturated blood contacts warmer tissues [7]. Technical constraints also limit CPB's application to the hospital setting, where it is available only after transport and operative delay. Less invasive modalities with potential for field use, such as surface cooling and cold saline lavage of body cavities, typically produce cooling at 0.10–0.15°C/min. The experimental technique of 'total liquid ventilation' (TLV) with chilled, oxygenated liquid perfluorochemicals (PFCs) uses the >20 m^2 surface area of the lungs for heat exchange, but thus far has been reported to cool little faster than surface techniques [8].

The ideal modality for rapid induction of systemic hypothermia would achieve cooling rates comparable to CPB, yet also be minimally invasive, easily implemented, and portable. With these goals we investigated a PFC lung-lavage technique combining some features of partial liquid ventilation (PLV) and cold saline lavage. At high PFC infusion rates and shorter cycle periods, the implementation of PFC lung-lavage begins to resemble TLV-cooling (or warming). In practice however, certain significant differences remain. In the technique we have termed 'gas/liquid ventilation' (GLV), the critical element of gas ventilation is retained. This gas ventilation component allows for flexibility in selecting ventilation parameters independently for heat and gas-exchange, and allows for liquid-mediated heat-exchange to be easily integrated into existing ventilation systems. It may also play a role in the surprisingly good thermal efficiency of GLV as compared with TLV.

The present study introduces GLV [9], explores the performance of GLV using a prototype automated liquid-delivery device, and finally discusses the basic mechanics and intrinsic limitations of heat-exchange using PFC lung-lavage.

2. Materials and methods

Trials described were approved by our Institutional Animal Care and Use Committee and were in compliance with the Animal Welfare Act and the National Research Council's Guide for the Care and Use of Laboratory Animals. Fifteen mongrel dogs weighing 13.8–25.7 kg were used (Table 1). Dogs were pre-medicated with I.M. acepromazine (1.0 mg/kg) and atropine (0.02 mg/kg) prior to induction of general anesthesia using sodium pentobarbital (30 mg/kg I.V., with maintenance dosing). Anesthetized dogs were intubated with a reinforced 10.0 mm I.D. (Willy Rüsch AG, Kernen, Germany) endotracheal tube (E.T.), and ventilated on room air using a Bennett MA1 or Siemens Servo 900 C ventilator. Ventilator parameters, unless otherwise noted, were 12 gas-breaths/min, gas tidal-volume of 15 ml/kg, I:E ratio of 1:3, and a maximal positive inspiratory pressure (PIP) limit of 26 cm H_2O (2.5 kPa). Gas pressures were measured at the E.T. adapter. Gas minute-volume ($\dot{V}_g$) was adjusted to maintain $PaCO_2$ between 35 and 40 torr. Animals were maintained at ~ 37.5°C prior to GLV, using a temperature-controlled water blanket. Rectal and bilateral tympanic temperatures (T_{tym}) were monitored continuously using a type-T thermocouple system (Cole-Parmer, Vernon Hills, IL) with a response time constant (t_o) of 5 s.

Combination pressure, blood sampling, and temperature-probe catheters were constructed from rigid polyethylene pressure-monitoring catheters, threaded centrally with 0.05 in. O.D. Teflon-sheathed type-T thermocouples ($t_o = 0.3$ s, Physitemp Instruments, Clifton, N.J.). In order to reduce the risk of catheter-as-

sociated clot formation, I.V. sodium heparin was given to adjust activated clotting times to 300–500 s, prior to central line placement. Femoral vessels were isolated surgically, and arterial and venous catheters placed and advanced to a level above the renal vessels, as confirmed by X-ray. During surgery, bupivacaine (0.5%) was infiltrated into wounds to mitigate post-operative pain. In one dog (Trial 1-2), a femorally-placed pulmonary artery thermodilution catheter replaced the venous combination catheter. Blood and ventilator pressures were acquired through a Hewlett Packard 78532-B monitor/transducer system.

2.1. Gas/liquid ventilation (GLV)

Immediately prior to GLV, dogs were assessed for adequacy of general anesthesia, then given Pancuronium Bromide (2 mg) to inhibit shivering and spontaneous breathing. FIO_2 was increased to 100% and external temperature control discontinued. To serve as a cannula for both infusion and removal of PFC liquid, a 19-Fr. flat-wire reinforced Bio-Medicus® venous catheter (Medtronic, Eden Prairie, MN) was introduced through the suction port of the E.T. adapter, and advanced ~45 cm to approximately the level of the carina (as confirmed by X-ray). This cannula was connected to the GLV apparatus described below.

GLV was performed using the PFC liquid 'FC-75' (3M Corporation, St. Paul, MN), a perfluorinated butyl-tetrahydrofuran isomer mixture [10,11]. A two-reservoir circuit (Fig. 1) was used to infuse and remove PFC from the lungs via the cannula, in cycle periods of 37 s (Trial I) or 16 s (Trial II). During timed PFC infusions ($t_{in} = 20$ s for Trial I, or 10 s for Trial II), PFC was pumped through the cannula by a continuously-engaged Travenol CPB roller-pump (Sarns, Ann Arbor, MI). A bypass loop, open during suction, allowed the roller-pump to divert (recirculate) PFC flow back into the storage reservoir whenever flow was not directed by line clamps V1–V3 into the animal. PFC was pumped continuously through an in-line 0.2 μm 'pre-bypass' filter, a primary heat-exchanger (Torpedo-T, Sarns, Ann Arbor, MI), and a combination silicone-membrane oxygenator/heat-exchanger (SciMed II-SM35, SciMed Life Systems, Minneapolis, MN). The oxygenator was supplied with 5–6.5 l/min O_2 (maximal device design rate), and the reservoir PFC was allowed to circulate and equilibrate with heat-exchangers and O_2, before GLV was initiated. The circuit tubing was constructed of S-50 HL TYGON® 3/8 and 1/2 in. I.D. class VI tubing (Norton/Performance Plastics, Akron, OH) save for a length of silicone tubing (Masterflex® 96410-73, Barrant Co., Barrington, IL) used in the roller-pump head in order to retain flexibility at cold temperatures. PFC suction was driven by a vacuum pump (model 107CAB18B, Thomas Compressors, Sheboygan, WI), and suction reservoir negative pressure was limited to −35 torr by a vacuum relief valve.

2.2. Gas/liquid ventilation protocol

Concurrent FC-75 lavage and gas ventilation (GLV) was performed for 18 min in Trials I and II ($n = 12$). This time was chosen, on the basis of preliminary work (data not shown), to achieve rapid systemic-cooling of greater than 5°C. For Trials I and II, the PFC recirculation rate within the GLV device (= PFC infusion rate, $\dot{V}_{inf}$) was set at 50 ml/kg per min. Immediately after a timed infusion of PFC into the lungs, PFC was removed as rapidly as the system vacuum allowed. Infusion of PFC for the next cycle began immediately after suction was discontinued. In 'cold' lavage experiments, PFC was chilled to ~4°C prior to lung infusion (Table 1), whereas in normothermic (control) dogs, isothermic PFC was delivered to the dog within ~2°C of tympanic temperature (T_{tym}). The PFC inflow and outflow temperature was measured continuously by a thermocouple inserted into the PFC path at the base of the delivery/removal cannula. Temperature data was collected throughout GLV, and for 22 min after GLV was completed. Arterial blood gas (ABG) samples were taken from the femoral arterial line before the start of GLV, and every 2 min during GLV. Following the post-GLV equilibration period, monitoring devices were removed and incisions closed.

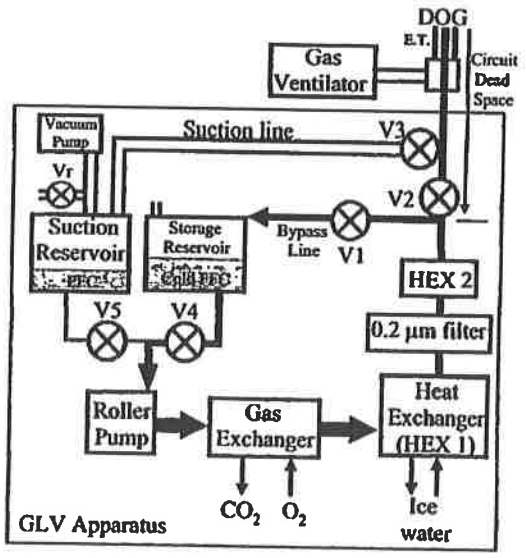

Fig. 1. The GLV lung lavage system. The GLV system was connected to a catheter inserted into the suction port of the E.T. adapter. Circuit PFC flows were directed by manual or mechanical clamps at V1–3. During the suction phase, PFC from the lungs was removed into a sealed 'suction' reservoir, for later addition to the primary circuit (via adjustment of V4 and V5), while 'infusion ready' PFC was re-circulated through a bypass loop. Negative pressure was limited by a vacuum relief valve (Vr).

Table 1
Summary of controlled variables in GLV[a]

	Dog mass (kg)	Initial tym. temp. (°C)	Total GLV duration (s)	PFC infusion temp. (°C)	No. of PFC cycles (n)	GLV cycle length (s)	Total PFC infused in GLV (ml/kg)	Flow in PFC infusion phase (V_{inf}) (ml/kg per min)
Trial I								
I-1 (cold)	20.7	38.5	1063	2.4	28	38	553	50.7
I-2 (cold)	16.4	37.6	1056	2.9	25	42	548	50.7
I-3 (cold)	22.0	38.6	1076	2.8	29	37	494	49.4
I-4 (cold)	25.0	37.7	1111	5.9	30	37	549	50.8
I-5 (cold)	18.7	38.4	1083	6.2	34	32	609	49.7
Mean	20.6	38.2	1078	4.0	29	37	551	50.3
SD	3.3	0.42	21	1.8	3.2	3.7	41	0.6
I-6 (warm)	16.9	37.7	1046	37.4	30	35	523	50.5
I-7 (warm)	21.5	36.7	1066	37.1	27	39	452	48.8
Trial II								
II-1 (cold)	19.0	36.9	1092	5.7	63	17	589	56.8
II-2 (cold)	13.8	35.7	1086	2.1	83	13	692	50.0
II-3 (cold)	18.2	36.4	1075	5.9	76	14	682	50.0
Mean	17	36.3	1084	4.6	74	15	654	52.3
SD	2.8	0.6	9	2.1	10	2.2	57	4.0
II-4 (warm)	17.5	37.1	1077	38.0	67	15	561	50.3
II-5 (warm)	17.6	36.1	1073	38.2	54	20	573	63.6
Additional experiments								
A[b] (cold)	21.8	37.0	600	4.4	1	600	76	32.4
B (cold)	25.7	36.8	6040	−0.3	15	403	354	6.07
C (cold)	24.5	39.7	4933	−0.5	21	235	682	6.00–16.7

[a] PFC, perfluorocarbon; GLV, gas/liquid ventiaaaaalation; tym., tympanic; temp., temperature; SD, standard deviation.
[b] Animal A was given the PFC FC-84 which has a heat capacity that does not differ significantly from FC-75.

Table 2
Summary of thermal results of GLV[a]

Trial	Maximum tym. ΔT (°C)	Net tym. ΔT_c (°C)	Total heat removed ΔQ_T (kJ/kg)	Mean heat transfer efficiency E_T	Mass-specific heat capacity C_m cal/g per °K (kJ/kg per °K)	Thermal dead space $V_{D\text{therm}}$ ml/kg
Trial I						
I-1 (cold)	−7.5	−6.3	21.9	0.68	0.83 (3.5)	5.9
I-2 (cold)	−7.8	−6.6	19.8	0.67	0.72 (3.0)	6.2
I-3 (cold)	−7.5	−6.2	19.3	0.63	0.74 (3.1)	6.9
I-4 (cold)	−6.9	−5.8	14.7	0.53	0.61 (2.5)	8.9
I-5 (cold)	−7.7	−6.2	15.4	0.50	0.59 (2.5)	9.5
Mean	−7.5	−6.2	18.2	0.60	0.70 (2.9)	7.5
SD	0.35	0.28	3.1	0.09	0.1 (0.42)	1.6
I-6 (warm)		+0.5	N/A	N/A	N/A	N/A
I-7 (warm)		+0.2	N/A	N/A	N/A	N/A
Trial II						
II-1 (cold)	−6.1	−5.1	22.6[b]	0.74[b]	1.1 (4.4)[b]	4.5
II-2 (cold)	−5.1	−4.3	23.8[b]	0.61[b]	1.3 (5.5)[b]	5.9
II-3 (cold)	−5.3	−4.2	19.0[b]	0.59[b]	1.1 (4.5)[b]	5.5
Mean	−5.5	−4.5	21.8[b]	0.65[b]	1.2 (4.8)[b]	5.3
SD	0.5	0.5	2.5[b]	0.08[b]	0.15 (0.61)[b]	0.7
II-4 (warm)		+0.4	N/A	N/A	N/A	N/A
II-5 (warm)		+0.2	N/A	N/A	N/A	N/A
Additional experiments						
A (cold)	−1.9	−1.5	4.71	1.0	0.75 (3.1)	0.0
B (cold)	−8.4	−7.3	19.4	0.89	0.63 (2.7)	2.6
C (cold)	−12.5	−12.1	33.8	0.84	0.66 (2.8)	5.2
Mean	N/A	N/A	N/A	N/A	0.68 (2.9)	N/A
SD					0.06 (0.26)	

[a] PFC, perfluorocarbon; GLV, gas/liquid ventilation; tym., tympanic; SD, standard deviation; N/A, not applicable.
[b] See text Appendix A.3.

2.3. Trial I (manually-controlled GLV)

Trial I was designed to investigate the variability in individual animal response to GLV, and to investigate the physiological effects of GLV delivered with and without thermal stress. Either isothermic (near-body temperature) or cold PFC lavage was administered using a manually-controlled system (V1–V5 in Fig. 1 represent CPB tubing-occluders in this Trial). One lavage cycle (period $t_c \sim 37$ s) was composed of a timed PFC infusion ($t_{inf} = 20$ s), followed by PFC suction ($t_s \sim 17$ s). Suction was stopped when PFC liquid return became sparse, or gas pressure in the ventilator circuit fell below -5 cm H_2O (-0.5 kPa). Five dogs received cold PFC (Trial I-1–5), while two controls received the same protocol using isothermic PFC (Trial I-6 and 7).

2.4. Trial II (machine-controlled GLV)

Trial II assessed the utility of using an automated device (custom manufactured by Korr Medical, Inc., Salt Lake City, UT) to perform rapid-cycle GLV. Computer-controlled solenoid clamp-valve occlusion of circuit lines at V1–V3 allowed smaller lavage volumes (V_{lav}) and smaller t_c. While t_{inf} was decreased to 10 s in Trial II, $\dot{V}_{inf}$ remained constant, and the effective PFC lavage rate ($\dot{V}_{lav}$) remained in the range of $\dot{V}_{lav}$ for Trial I. Table 1 gives relevant trial parameters. In Trial II, suction removal of PFC from the lungs began immediately after infusion, and was automatically stopped whenever ventilator circuit pressure of -5 cm H_2O was reached ($t_s \sim 6$ s, giving $t_c \sim 16$ s). Three dogs received cold PFC (Trial II-1–3), while two controls (Trial II-4 and 5) received isothermic PFC.

2.5. Animals A, B and C

Selected data from three dogs in an earlier method-development series was used. These dogs had been prepared as above, then manually given 1, 15 and 21 lavages, respectively with cold PFC, at much slower rates than in Trials I and II (Table 1). Data from these animals allowed independent measurements of lavage-volume heat-contents and temperatures, and thus heat

capacities and heat transfer efficiencies, by a more thorough thermal accounting method (Table 2, Appendix A).

2.6. Data collection and correction, statistical methods, graphical display and presentation

Temperature and pressure data were collected using a PCI E series data acquisition board and LabView™ software (National Instruments, Austin, TX). Graphical analysis and display of temperature data, and curve fitting, was done using the software package Origin™ (Microcal Software, Northhampton, MA). Statistical comparison of Trial group values was done using GraphPad Prism (GraphPad Software, San Diego, CA). Group means are reported ± standard deviation (SD) except as otherwise noted. For each animal, the T_{tym} from whichever probe cooled most rapidly, was used (right probe in 12/15 dogs). In order to facilitate comparison of cooling rates between sites in the same animal, temperatures at all probe sites were corrected to the baseline aortic temperature (T_{art}), as measured immediately prior to the start of GLV. For ease of description, GLV-cooling is presented in terms of thermal-deficit ('cold') moving from the lungs into successive body compartments. A compartmental analysis of thermal transfer in this model, and a glossary of notation and equations used, is given in Appendix A.

3. Results

The GLV technique allowed cyclic liquid lung-lavage of dogs undergoing concurrent gas ventilation. Suction from a submerged catheter tip at the carina allowed direct distal collection of PFC even during forced gas inspiration. We found that a long suction catheter was necessary to insure that higher suction pressures could be used to directly withdraw the dense PFC throughout the liquid removal phase, without prolonged exposure of the gas filled portion of the airways to the negative pressure of the suction system/reservoir. The relief valve limited negative pressures in the suction reservoir, and also in the lungs, for the relatively brief time after liquid flow no longer blocked the suction line. Suction in this manner was efficient, although lavage volume measurements showed that the lungs chronically retain ~12 ml/kg PFC (approximately the pulmonary residual capacity).

The PFC pump circulation/infusion rate ($\dot{V}_{inf}$), measured volumetrically preceding and following GLV, was stable to within 1% over the duration of GLV, and was not significantly different between trials ($P = 0.28$). The $\dot{V}_{lav}$, calculated as $t_{inf}\,\dot{V}_{inf}/t_c$, was 30.7 ± 2.3 ml/kg per min (Trial I) and 36.4 ± 3.2 ml/kg per min (Trial II). The $\dot{V}_{lav}$ was significantly ($P = 0.023$) larger in Trial II because machine-controlled suction made more efficient use of available non-infusion time, resulting in faster net PFC removal.

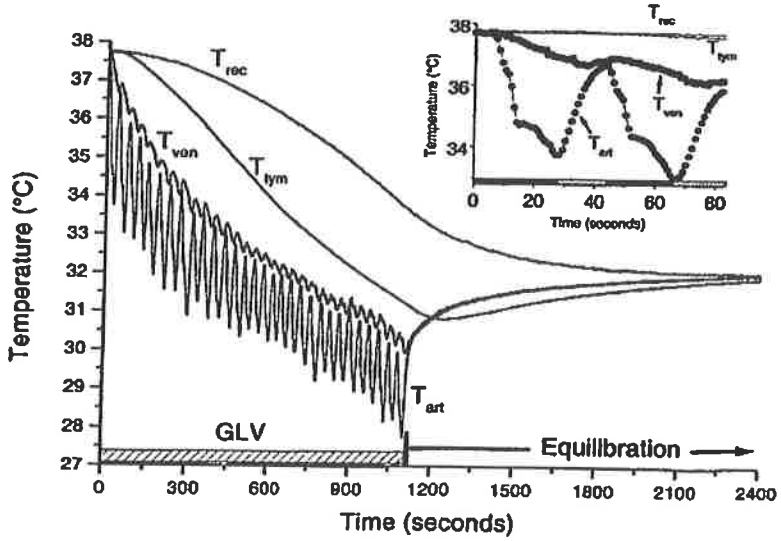

Fig. 2. Body temperature changes observed during GLV (Method of Trial I). In this illustrative experiment from Trial I (I-4), lavages of cold (4°C) FC-75 were infused (~ 20 s) and removed (~ 17 s) from the lungs. GLV was performed for 18 min (hatched bar), then stopped to allow thermal equilibration (22 min). Arterial temperature (T_{art}), central venous temperature (T_{ven}), tympanic temperature (T_{tym}), and rectal temperature (T_{rec}) are shown. *Inset:* Enlarged view of temperature changes recorded during the first two cycles of PFC infusion (dark bar) and removal (hatched bar).

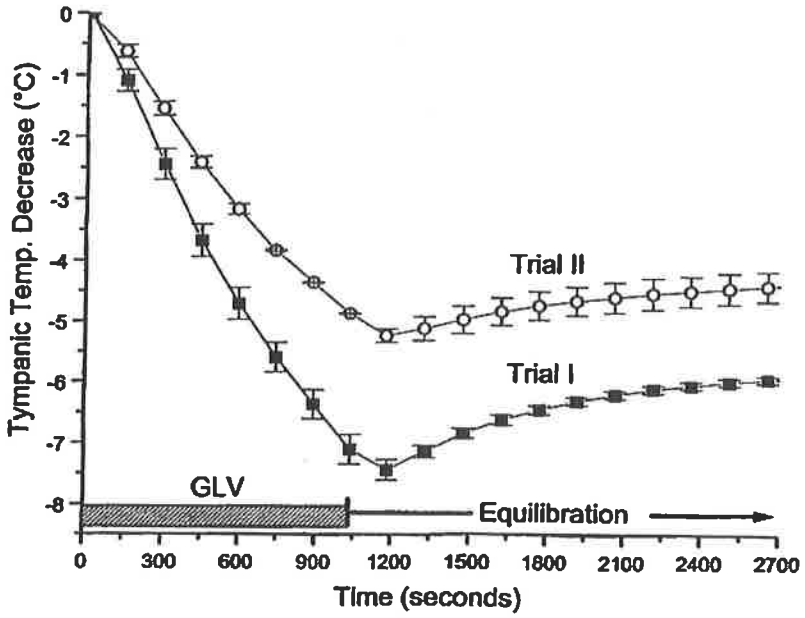

Fig. 3. Body temperature changes during manual and mechanical GLV (Trial I vs. Trial II). The relative rates of core body cooling in dogs undergoing 18 min (hatched region) of manual (Trial I, solid squares) or machine-driven (Trial II, open circles) cold GLV, were assessed by comparing changes in group mean T_{tym}. Symbols represent the mean and SEM ($n = 5$ for manual, and 3 for machine groups).

3.1. Thermal results of GLV

3.1.1. Cooling time delay

Fig. 2 illustrates GLV cooling in a representative dog (I-4) from Trial I. The T_{art} began to decrease 3–6 s after the start of each PFC lavage. Since this delay included circulation delay from lungs to aorta, the transfer of thermal-deficit from newly-introduced PFC to pulmonary blood was very rapid.

The venous temperature (T_{ven}) began to decrease 10.4 ± 6.9 s after T_{art} decline, representing the minimum systemic circulation time. Though exhibiting delay, damping, and broadening behavior (presumably due to peripheral heat-exchange and varying systemic blood-return path lengths), T_{ven} transients from lavages mirrored T_{art} transients. T_{tym} temperatures, presumably reflecting brain and viscera temperatures, were non-oscillatory.

The T_{tym} did not begin to decrease until ~ 24 s after the start of GLV. This decrease occurred in three phases: an initial phase lasting ~ 100 s, an exponential phase lasting for ~ 900 s, and a final linearly-decreasing phase lasting until the end of GLV. Core cooling as measured by T_{tym} continued for about 120 s after the end of GLV (Fig. 3), then exhibited a marked rebound effect [12] with exponential dampening ($t > 20$ min, Figs. 2–4). These phases of cooling and equilibration were consistent with a five-compartment thermal model, in which the three compartments representing animal tissues corresponded roughly with (1) the blood and vasculature; (2) the classical thermal core; and (3) the classical thermal periphery (Fig. 5). Modeling equations and estimation of compartment sizes are given in the Appendix A.

3.1.2. Cooling rate

Crude cooling rates were determined numerically from appropriate T vs. t graph segments. The mean cooling rate from GLV initiation, or $\Delta T_{tym}/\Delta t$, reached a maximum value in Trial I at -0.49 ± 0.09°C/min ($t = 6.6$ min). The differential cooling rate $d(\Delta T_{tym})/dt = dT_{tym}/dt$ reached a maximum (max) value of -0.59 ± 0.13°C/min at $t \cong 100$ s, near the end of the initial heat exchange development region. (This value is comparable to analytic $d(\Delta T_{tym})/dt$ (max) from (Eq. (1)) $= \Delta T_k/t_o = -0.63$°C/min). Corresponding cooling rates in Trial II were $\Delta T_{tym}/\Delta t$ (max) $= -0.33 \pm 0.02$°C/min (at $t = 7.3$ min) and dT_{tym}/dt (max) $= -0.37 \pm 0.06$°C/min (at $t = 100$ s).

3.1.3. Mean cooling power

The mean heat removal rate (cooling-power) P over the entire duration of GLV, for each animal, was estimated from ΔT_e according to $P = m\ C_m\ \Delta T_e/t$ (total). Here t (total) is the entire GLV application time $= \sim 1080$ s. (Note: for this calculation, the more accurate Trial I mean C_m is used for all Trial II animals.) The mean cooling power of Trial I was 336 ± 60 watts, while that of Trial II (using the Trial I value of C_m) was 207 ± 49 watts ($P = 0.02$). Variation in animal size was the major source of intra-group variability.

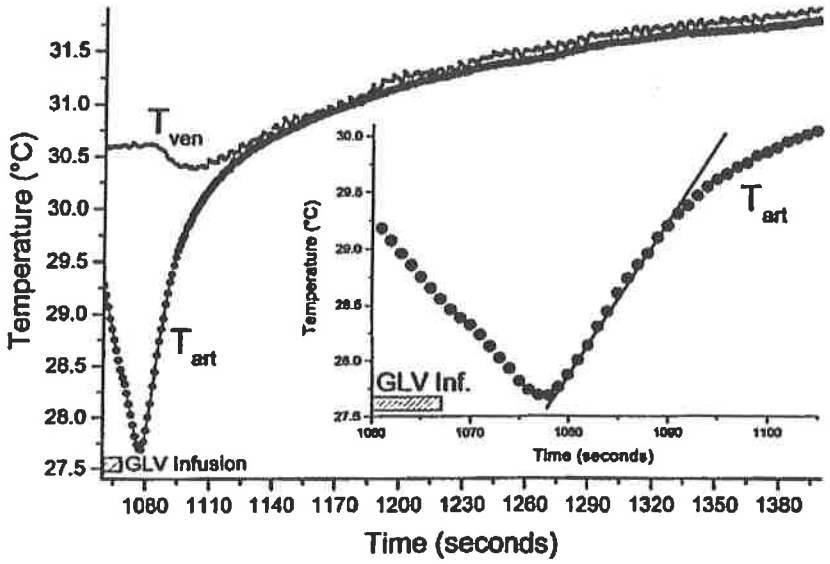

Fig. 4. Thermal equilibration after GLV. Mean T_{art} and T_{ven} values (Fig. 2) are shown for Trial I, dogs 1–5. To highlight equilibration changes, T_{art} curve nadirs ($n = 5$) were superimposed before calculation of means, and T_{ven} data ($n = 4$) for each dog was adjusted with its corresponding T_{art} curve. Incompatible T_{ven} data from a pulmonary artery thermodilution catheter in I-2 has been omitted. *Inset:* The sigmoidal mean ($N = 5$) T_{art} recovery during the first ~12 s after final GLV infusion halt is approximated by linear fitting.

3.2. Gas exchange

ABG measurements demonstrated that infusion of cold PFC stabilized PaO_2 and $PaCO_2$ during GLV. In contrast, GLV using isothermic PFC failed to maintain pre-treatment PaO_2 or $PaCO_2$ levels (Fig. 6). In Trial II-4, hypercapnia during the first 13 min of isothermic GLV was abolished by increasing the tidal volume from 15 to 25 ml/kg (final $\dot{V}_g = 375$ ml/kg per min). In Trial II-5, $\dot{V}_g$ was pre-set to 375 ml/kg per min in an attempt to avoid hypercapnia, and no significant ABG changes were observed.

3.3. Clinical observations and gross pathology

With the exception of one dog, animals subjected to GLV displayed mild tachypnea and increased expiratory sounds, but otherwise exhibited unremarkable recovery from anesthesia, including the ability to walk and drink. The exception was an eosinophilic animal (Trial II-1) which had normal oxygenation during GLV, but developed severe hypoxia shortly after GLV. Chest X-ray pre- and post-procedure showed no comparatively remarkable features. This dog was sacrificed at 9 h. Necropsy revealed a mass of *D. immitis* (heart-

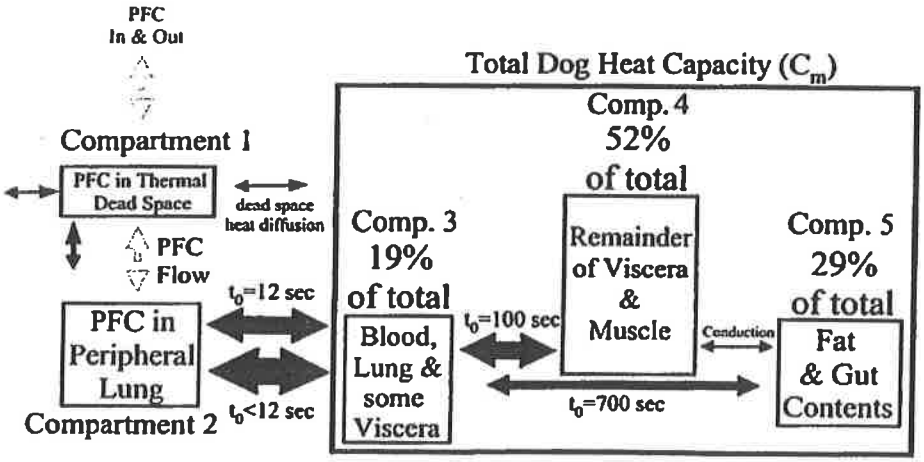

Fig. 5. Heat transfer among body compartments during GLV. Heat transfer during GLV in the dog may be modeled using 5 thermal compartments. Heat transfer between compartments (which is by blood circulation, except as noted) is shown in the box diagram as double-headed arrows. The pair of arrows connecting Compartments 2 and 3 represent the different processes of lung equilibration with (1) pulmonary artery flow; and (2) with the complete blood volume and selected viscera.

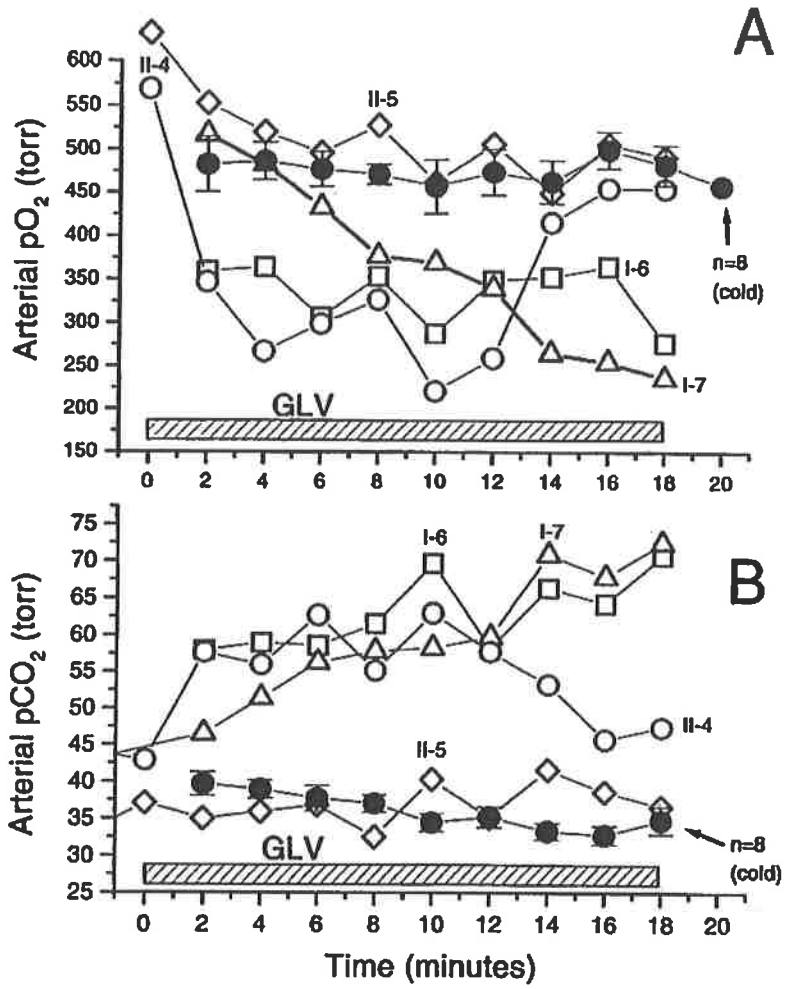

Fig. 6. GLV does not maintain normocapnia at isothermic temperatures without altering gas ventilator parameters. Animals in Trials I and II underwent GLV using either cold (closed symbols) or isothermic (open symbols) FC-75. Both arterial PaO_2 (Panel A) and $PaCO_2$ (Panel B) levels were affected by lavage temperature. Cold lavage data from Trials I and II were very similar in magnitude, and therefore, have been combined ($n = 8$). Isothermic GLV is shown as four separate experiments (Trial I-6 and 7, and Trial II-4 and 5). Gas tidal volume was increased from 15 to 25 ml/kg in Trial II-4 at $t = 13$ min, and at $t = 0$ in Trial II-5, normalizing PaO_2 and $PaCO_2$ in both animals. Declining PaO_2 in Trial I-7 was due to inadvertent failure to pre-oxygenate PFC.

worm) embolized into the pulmonary arterial circulation, possibly as a result of cold GLV (this animal had been heartworm seronegative). Necropsies performed on nine remaining Trial I and II animals sacrificed 24 h post-procedure revealed diffuse spongy, resilient 'foam rubber' lung lesions typical of lungs exposed to a high-vapor pressure PFC at high PIP pressures. This was noted especially in anterior portions of lung lobes. This trapped intraparenchymal PFC was thought to be the cause of broncho-constriction and wheezing found typical of post-GLV animals. There was gross-level evidence of dependent-lung damage with alveolar-filling in both isothermic and cold PFC-lavaged animals. Other organ systems in this series were grossly normal. Two animals in Trial II (II-3 and II-4) were not sacrificed, and were held for long term evaluation. They were neurologically normal at 1 year post-GLV.

4. Discussion

4.1. Apparent effect of temperature on gas exchange

Isothermic GLV in our model was surprisingly poor at removing CO_2, considering that the CO_2 carrying capacity in FC-75 decreases by only ~23% from 0 to 40°C (extrapolated from [11]). A useful observation was that even pO_2 values decreased in isothermic animals, indicating an extreme influence on total ventilation. Capnographic analysis of GLV in Trials I and II (data

not shown) indicated that isothermic GLV had a much larger negative effect on pressure-limited total gas ventilation $\dot{V}_g$, as compared to cold GLV using the same technique and the same gas ventilator settings. Since GLV at a $\dot{V}_{lav}$ of 30–36 ml/kg per min relies on gas ventilation $\dot{V}_g$ for >50% of total alveolar ventilation, a differential loss of pressure-limited $\dot{V}_g$ with temperature appeared to be the basis of CO_2 retention in isothermic GLV. The mechanism of the implied differential change in lung compliance is unclear. However, gas ventilation adjustments similar to those in Trial II- 4 and 5 may be required if GLV is used as a re-warming technique.

4.2. Thermal transfer efficiency and kinetics

The optimal GLV cooling (or warming) protocol remains unknown. However, the finding that the thermal equilibration of non-dead space PFC and local pulmonary blood flow proceeds very rapidly ($t_o < 12$ s) suggests that PFC liquid infusion times need to be no longer than this time scale. When PFC-bolus lung residence times exceed this duration, the lavage bolus is in place longer than is required to transfer the most labile part of its thermal potential to the pulmonary blood and parenchyma. Since PFC ventilation rates ($\dot{V}_{lav}$) in the present study are already at least a third of the maximal rates possible in TLV, it seems probable that PFC infusion rates and pressures, rather than heat transfer rates from PFC to lung, will be the fundamentally limiting factor to power transfer in GLV. Our observations suggest that, as least to $\dot{V}_{lav}$ rates of 30 ml/kg per min and $\dot{V}_{inf}$ rates of 50 ml/kg per min, the total cooling power (cooling rate) in GLV will be greatest if no lavage dwell time is allowed, and all available time during the respiratory cycle is used to either introduce, or remove, PFC.

4.3. Question of diffusion dead space in GLV

Mammalian lungs depend on simple gas diffusion for CO_2 transport through acinar airways during normal tidal ventilation. An intractable problem in experimental TLV has been that simple diffusion is not sufficient to similarly move CO_2 through liquid PFC at physiologic CO_2 partial pressure gradients. This limitation appears physiologically in liquid ventilation as a 'CO_2 diffusion dead space' which effectively lowers alveolar ventilation. In part due to such extra physiologic dead space, TLV of adult humans has been estimated to require liquid minute-volumes near 70 ml/kg per min [14]. This value is at the upper bound of realistically attainable liquid flow rates [15,16], and leaves little leeway for treating hypermetabolic or acidotic states, or lung disease. Such difficulties are not a theoretical limitation in GLV, however, since GLV does not require high liquid flow rates for ventilation. In the most rapid-cooling GLV protocol used in this trial, $\dot{V}_{lav}$ was 31 ml/kg per min—a low baseline value which permitted the addition of 10 times this minute-volume of gas ventilation (see Fig. 4, Trial II-4,5). Moreover, since normal gas minute volumes were required to maintain normocapnia in Trial I, there is as yet no evidence in GLV for any CO_2 diffusion limitations caused by PFC in the lungs. Possible reasons for this are discussed below.

Thermal-diffusion limits in TLV have not been studied per se, but their presence is suggested by the results of Shaffer and coworkers [8]. In the cat TLV model using a $\dot{V}_{lav}$ of 75 ml/kg per min, a decrease in PFC inspiration temperature from 20 to 10°C (increasing the thermal gradient by a factor of 1.6) increased the cooling rate from −0.13 to −0.15°C/min. This small rate change represented a significant loss of efficiency. By contrast, in the present GLV study using PFC at 4°C, there was no evidence of a thermal-diffusion limit at rates up to 4 'liquid-breaths'/min. Notably, in Trial I, where 100% of the V_{lav}, and 40% of the $\dot{V}_{lav}$ of the cat TLV model was used, cooling rates for GLV were more than three times those reported for cats on TLV at 4.5 liquid breaths/min at 10°C [8].

The possible quantitative presence of a thermal diffusion limit for GLV at 4 liquid breaths/min may be evaluated using a modified version of the concept of gas-exchange dead space (V_D). The respiratory system of a dog undergoing GLV heat-exchange may be considered, by analogy with gas exchange dead space (V_D), to also contain a 'thermal exchange dead space' (V_{Dtherm}). Each thermal lavage volume V_{lav} (analogous to a liquid breath) of PFC then also contains a V_{Dtherm}, which by definition does not participate in heat-exchange. Thus, cycle thermal transfer efficiency E_f may be expressed as $(V_{lav} - V_{Dtherm})/V_{lav}$, and any measured value of mean E_f may be expressed as an equivalent mean $V_{Dtherm} = V_{lav}(1 - E_f)$. For Trial I ($E_f = 0.6$, Appendix A for calculation), mean V_{Dtherm} was then seen to be 7.5 ± 1.6 ml/kg, and in Trial II (using Eq. (6) E_f value = 0.40), V_{Dtherm} was 5.3 ± 0.8 ml/kg ($P = 0.072$). The absence of an increase in V_{Dtherm} in Trial II vs. Trial I indicated that the size of V_{Dtherm} in these GLV protocols was non-dynamic at time-scales of one lavage period, providing evidence against the presence of a 'thermal diffusion dead space' (analogous to a CO_2 diffusion dead space) at these lavage rates.

In absolute terms, it may be useful to compare calculated V_{Dtherm} in the GLV dog model to the expected physiologic gas-exchange dead space, V_{DCA}, which in healthy animals is close to the dog anatomic $V_D = \sim 6.5$ ml/kg [17]. In thermal diffusion as for gas diffusion, diffusion physiologic dead space would be expected to significantly add to anatomical dead space. However, the sum of mechanical-V_D in the GLV circuit (~ 1.5 ml/kg) plus the anatomic V_D for dogs, is found

to be more than the calculated V_{Dtherm} in either trial in this study, leaving little room for a large heat-diffusion contribution to V_{Dtherm}. For these reasons it is suggested that the loss of cooling power observed in Trial II was not due to heat diffusion limitations, but instead due to a loss of efficiency effect similar to that seen with low tidal volumes in ordinary gas ventilation. In these terms, low lavage volumes in GLV result in an increase in 'thermal dead space ventilation' at the expense of PFC flow involved in active heat-exchange, resulting in a larger 'wasted' lavage fraction V_{Dtherm}/V_{lav}.

V_D in heat transfer (V_{Dtherm}) is analogous to V_D in gas-transfer, in as much as all dead space is 'diffusion dead space' at long-enough time-scales. However, some of the mechanisms for diffusion modification of V_{Dtherm} are unique. By contrast with gas molecules, heat diffuses rapidly through device tubing into PFC in the GLV circuit dead space, and also diffuses directly through the tracheal wall into the anatomic-V_D. Thus, heat diffusion from dead space liquid at sufficiently slow lavage rates might be expected to have a pronounced effect on E_f in GLV, due to slow heat-diffusion reduction in V_{Dtherm}.

Some evidence for such a process was found, though at lavage dwell times too long to be of interest for rapid cooling. At the relatively small t_e of Trials I and II, the calculated V_{Dtherm} was found to be $\sim V_{DCA}$; but in animal B, with a much longer t_e of 7 min, the V_{Dtherm} was only 2.6 ml/kg. The limit of this process was reached in animal A, in which the V_{Dtherm} of a single retained 'breath' of highly-oxygenated PFC fell to nearly zero after 10 min. Disappearance of V_{Dtherm} by thermal equilibration, estimated from individual cycle E_f variations in animals B and C, was estimated to occur with a half-time of ~ 5 min (data not shown). This process was slow enough to be neglected when GLV lavage periods (t_e) were less than several minutes. Thus, at the lavage rates of Trials I and II, a full-sized V_{Dtherm} of ~ 6 ml/kg appeared, and accounted for significant loss of cooling power at low V_{lav} (e.g. Trial II where V_{lav} was only 8.8 ml/kg).

The characteristic size of V_{Dtherm} at all but the slowest lavage rates (< 1 lavage per 5 min) implies that the only thermally-efficient solution for performing GLV at faster rates is maintenance of $[V_{lav}/V_{DCA}]$ or $[V_{lav}/V_{Dtherm}]$ ratios > 3, in order to avoid excessive 'wasted' V_{Dtherm} ventilation. This requires a V_{lav} of ~ 18 ml/kg in dogs. In humans, where anatomic-V_D is < 3 ml/kg, less than half the value for dogs, both the V_{Dtherm}, and therefore, most-efficient V_{lav} values, might also be expected to be correspondingly less. In any case, it is clear that rapid-cooling GLV techniques cannot wait for the relatively slow thermal equilibration of PFC within the anatomic V_D, since equilibration in the remaining non-V_D parts of the lung is so rapid (i.e. less than Trial II t_e of 16 s).

4.3.1. Possible synergy of combined gas and liquid ventilation in assisting mass (CO_2) and heat transfer

The absence of expected heat-diffusion and gas-diffusion limitations in GLV suggests that some assistive process for both gas and heat transfer through PFC in the peripheral lung may occur in GLV. The authors' fluoroscopic observations (made with the non-brominated and relatively radiolucent FC-75) have been that each gas breath in PLV produces a flash of fine bubbles which spread uniformly throughout the lung. As compared to the more familiar behavior of water, the low surface tension of PFCs (15 dyne-cm for FC-75, about 1/5th that of water) lowers the energy barrier to producing small bubbles in forced gas/liquid flows. Such bubbles moving within small airways may induce eddies and turbulence in laminar liquid flows at small scales, contributing significantly to heat and mass (CO_2) transport though PFC liquid by means other than diffusion. We hypothesize that the lack of bubble-induced turbulence in TLV may account for the large diffusion-dead-space for heat and CO_2 which seems to be present in TLV at even low breathing rates — an effect which is apparently absent in both PLV and GLV.

4.4. Potential development of clinical GLV

Rapid brain cooling has become a goal of resuscitation research. Based on their work, Safar et al. have noted that clinical implementation of mild resuscitative hypothermia, which is effective in large animal models, will depend on the development of rapid mild brain cooling methods [18]. A recent editorial in 'Stroke' [6] commented on the striking ability of combination mild hypothermia and pharmacological pre-treatment to ameliorate ischemic brain damage in the cerebral artery occlusion rat model, then addressed similar concerns:

> A problem for use of this technique for acute stroke therapy is that the time required to induce hypothermia in patients is likely to be considerably longer than for rats. [...]. To substantially increase the rate of hypothermia induction in humans, it will almost certainly be necessary to use some sort of invasive procedure, such as a heat-exchanger, to cool the circulation.

The technique of GLV may eliminate the need for such invasive measures. For example, in the cited trial [19], rats were cooled from 37 to 33°C (-4°C) over 40 min, using external ice packs. By contrast, the present study finds cooling of the canine body core and brain by -4°C in less than 10 min.

Development of clinical GLV awaits identification of suitable PFCs for various GLV applications. For example, the pharmaceutical PFC perfluorooctylbromide

(Perflubron®, Alliance Pharmaceuticals) would presumably not be suitable for fast GLV cooling due to its freezing point of +3°C, but might be useful for slower cooling or for GLV re-warming. Some industrial PFCs have pour-points low enough to make them potentially useful as GLV rapid-cooling media; however, some of these also have unsafely-high vapor pressures at 37°C. Such low-boiling point PFCs apparently exacerbate barotrauma injury by adding intraparenchymal PFC-vapor damage to lung pathology. They may also increase the danger of lung compression due to escaped PFC entering the pleural space and vaporizing (so-called 'fluorothorax'). FC-75, (formerly named FX-80) is historically the oldest of the PFC liquid ventilation media [10], but its relatively high vapor pressure probably makes it a less than optimal GLV agent.

Assuming that a PFC with the correct biophysical properties is identified and produced to medical standards, GLV should be easily scalable to the human adult. For example, the viscosity of FC-75 is similar to water [11], and under standard suction a 19 Fr. adult pulmonary toilet catheter will remove FC-75 at ~2 l/min. As in the system described, a GLV system may interface with a conventional gas ventilator system via a simple liquid-carrying catheter which extends through the endotracheal tube adapter suction port.

5. Conclusions

GLV is capable of inducing hypothermia in a fraction of the time that it takes to prepare a patient for cooling via CPB. In addition, automated GLV need not have the spatial and technical restrictions of the hospital setting. Although relatively simple pumpless methods of continuous arteriovenous shunt heat-exchange have been described which might be potentially applicable in the field [20], even these have the drawback of requiring skilled cannulation of a major artery. Since the primary technical skill required to initiate GLV in the field would be endotracheal intubation, GLV by contrast may be a candidate for a much wider range of emergency field-uses in civilian and military settings. GLV holds promise for central warming in severe hypothermia, although an absolute maximal PFC temperature of 42°C would in theory limit the re-warming rate to about one-third of that possible in cooling. GLV has potential as a very rapid treatment for heatstroke and malignant hyperthermia. Whether used inside or outside hospitals, successfully implemented GLV might serve more generally as a neuroprotective bridge [21] in order to gain time for more technical, supportive, or definitive treatment (e.g. neurovascular clot disruption, emergency CPB, cord decompression or hemorrhagic shock/trauma surgery).

Although certain types of liquid breathing are being clinically tested [22], the safety parameters of rapid and cold liquid delivery to the lungs remain to be determined. As noted in this study, GLV can cause lung damage. While the mechanism of such damage is unclear, the placement of lesions suggests that both barotrauma (dependent lung) and volu-trauma (non-dependent lung) may occur. We have generally observed that GLV causes little permanent lung damage in long term survival models. Similar pathology seen in lungs exposed to either isothermic or cold GLV in the present study (data not shown) suggest that thermal/chilling-injury per se is not the major insult in such gross damage. Although more subtle lung biochemical and immune problems (pneumonia) from hypothermia itself are suggested by reports from some longer duration therapeutic hypothermia studies [23,24], it is not clear that the minimal duration treatment necessary for effect in post-resuscitative hypothermia greatly pre-disposes to such problems. We hypothesize that lung pathology in GLV may be reduced with better control of GLV pressure and volume limits, and by use of PFC liquids having more physiologically suitable properties.

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Appendix A

A.1. Abbreviations and notation

In the text, volumes (V) are given in ml/kg, and flows ($dV/dt = V' = \dot{V}$) in ml/kg per min. Since all V and $\dot{V}$ are expressed in mass-specific (per kg animal) terms, derived quantities ΔQ and C_m are automatically mass-specific. C_m and C_{vf} are given in calories/(g or ml) per °K for easy comparison with water.

PFC: Perfluorochemical. Hydrogen-free organic molecule in which most peripheral atoms are fluorine. *TLV:* Total liquid ventilation. Modality in which only liquid fills the lungs and ventilator. *PLV:* Partial liquid ventilation. Modality in which all gas exchange is via gas ventilator, with ~1/2 FRC of PFC liquid residing in the lungs to help open dependent alveoli. *GLV:* Gas/liquid ventilation. Heat-exchange modality in which ventilation is by both gas ventilator and PFC lavage.

T_{tym} tympanic temperature
T_{art} arterial temperature
T_{ven} central venous temperature
T_{rec} rectal temperature
ΔT_c net ΔT_{tymp} resulting from GLV, after equilibration at $t = 40$ min
t_{inf} lavage cycle infusion time
t_s lavage cycle suction time
t_c lavage cycle period ($= t_{inf} + t_s$)
V_{lav} single-cycle PFC lavage infusion volume = $t_{inf}\,\dot{V}_{inf}$
V_S single-cycle PFC lavage suction-return volume
V_D ventilatory dead space (any type)
V_{DCA} expected gas ventilation V_D = sum of circuit (mechanical) V_D plus anatomic V_D
V_{Dtherm} thermal or heat-exchange V_D (ml/kg, in reference to liquid PFC infusion)
$\dot{V}_{inf}$ PFC infusion rate (set to $\cong 50$ ml/kg per min in Trials I and II)
$\dot{V}_{lav}$ effective PFC lavage rate = GLV liquid minute-ventilation (ml/kg per min) = V_{lav}/t_c
$\dot{V}_g$ gas minute-ventilation (ml/kg per min)
m animal mass
C_h heat capacity
C_T total heat capacity of the animal ($= mC_m$)
C_m mean mass-specific heat capacity of the animal ($= \Delta Q_T/\Delta T_c$)
C_{vf} volume-specific heat capacity of FC-75 (mean of 0 and 25°C values) = 0.45 cal/ml per °K
ΔQ_T total heat removed during GLV (kJ/kg animal) = $\Sigma\,\Delta Q_c$
ΔQ_c heat removed during one lavage cycle
E_f mean cycle heat transfer efficiency = mean of [ΔQ_c/(theoretic ΔQ_c (max)] for all cycles in a single experiment
n number of lavage cycles in GLV experiment
Σ sum entire quantity following, for all cycles $i = 1$ through n
T_{inf} PFC infusion temperature
T_S PFC suction removal temperature (time-averaged PFC suction flow temperature)
T_{SM} PFC mixed suction return-volume temperature (temperature of mixed V_S)

A.2. Thermal kinetics

During GLV cooling and equilibration, the blood and tympanic temperature changes in the animals were modeled by a simple five compartment model (Fig. 5). During the initial ~100 s of GLV (value used as empiric time mark), full development of heat-exchange behavior is established between the lungs, blood volume, and the thermal core of the animal, as suggested by the characteristic half-times for equilibration of these systems (see below).

Modeling of cooling during GLV: After the initial ~100 s of cooling, the data for tympanic $\Delta T(t) = \Delta T_{tym}$ during GLV in Trial I and II were modeled by a single time-constant exponential decline. Mean ΔT_{tym} data for each trial from times $t = 100$ to 1080 s were fit using (Eq. (1)).

$$\Delta T(t) = T_{100} + \Delta T_k[1 - \exp(-t/t_o)], \quad (1)$$

$\Delta T(t)$, total T_{tym} change from baseline T_{tym} at start of GLV; t, = time in seconds after empiric time mark, 100 s after start of GLV; T_{100}, observed ΔT at empiric time mark, 100 s after start of GLV; ΔT_k, observed temperature-interval constant, specific to each GLV method; t_o, observed natural-base time-constant, in sec (t_o = half-time/ln 2).

Best-fit values for Trial I data were: $T_{100} = -0.52 \pm 0.02$°C; $\Delta T_k = -11.2 \pm 0.02$°C; and $t_o = 1064 \pm 3$ s. Trial II values were $T_{100} = -0.24 \pm 0.02$°C; $\Delta T_k = -8.14 \pm 0.02$°C; and $t_o = 1107 \pm 5$ s. The relatively long time-constant associated with this thermal phase, which was similar in the two trials, presumably reflects the long time-constant (~700 s, see below) associated with heat transfer from the thermal core of the animal to the thermal periphery; thus the exponential phase represents full development of heat exchange between the GLV cooling device and the entire animal. The final linear segments of cooling occurring after this phase, measured at −0.29°C/min (Trial I) and −0.21°C/min (Trial II), represent the final relatively simple state which exists after heat exchange equilibrium between cooling device and animal has been fully established.

Cooling in blood and tympanic sites during GLV, and thermal evolution in these sites during equilibration phase after GLV was discontinued, was in accordance with a five-compartment thermal model (Fig. 5). In this model, the tissues of the animal are divided into three thermal compartments, corresponding loosely with the vascular system, the thermal core, and the thermal periphery.

Modeling of equilibration after GLV: Circulatory forced-convection is the major heat transfer mechanism in very rapid whole-body cooling processes. This fact allowed T_{art} and T_{ven} changes to be used to quantitate some features of heat transfer between body thermal compartments during the equilibration period after GLV. The mean T_{art} curve in Trial I increased nearly linearly ($R^2 = 0.9976$) for 12 s after the end of GLV, rising at a rate of 7.9°C/min. After this initial 12 s, T_{art} departed from linearity (Fig. 4, inset), and was modeled by the sum of three exponential terms with respective time constants (t_0) of 12 ± 0.4, 102 ± 2 and 701 ± 8 s. These t_0 times differed to a large enough extent that

their respective influences could be considered to be controlling over discrete time periods of about twice their value. Thus, the four *equilibration phases* seen after the end of GLV lasted approximately 12, 24, 200, and 1400 s (23 min), respectively and represented 34, 14, 25, and 27% of the 5.1°C rise in T_{art} during equilibration after GLV.

These data may be interpreted as follows: during each phase of the equilibration process, one or more thermal compartments in the animal equilibrated with the next-most closely-connected compartment (Fig. 5). Afterwards, the newly captured compartment(s), as part of a larger unit bound together by blood convection, equilibrated with the next-most closely connected compartment, and so on. The 12 s linear *first equilibration phase* (Fig. 4, inset) most likely represents development of heat transfer from lungs to local pulmonary blood flow. This phase was not associated with blood recirculation since it was seen as a rise in T_{art} but not T_{ven}. The *second equilibration phase* (duration $\sim$24 s) was characterized by an increase in dT_{ven}/dt to the value of dT_{art}/dt, indicating that the lungs, blood-volume, and certain other well-perfused viscera, such as the kidneys, were now evolving into a single thermal system. Since the observed t_o for this phase was 12 s, less than the animal's mean circulation time (= cardiac output/blood volume $\cong$30 s), this process appeared to be driven by blood circulation via the most rapid paths (e.g. renal circulation). Such short paths for circulatory heat transfer were evident in the relatively small lag times (10.4 $\pm$ 6.9 s) noted between T_{art} and T_{ven} changes in these animals.

During the first two equilibration processes, the pulmonary circulation added thermal potential to the blood-volume more rapidly than it could be removed by the entire systemic circulation. By the end of the *second equilibration phase*, however, lung-to-blood heat transfer no longer dominated, and the gap between T_{art} and T_{ven} was set by the magnitude of heat transfer from the blood-volume to remaining 'thermal core' systemic tissues. In this *third equilibration phase* (duration $\sim$200 s), the viscera and blood-volume, as a unit, equilibrated with the remainder of the 'well-perfused' tissues of the body (thermal core, comprising about 70% of the animal's heat capacity). Heat capacities for thermal compartments are calculated below. The t_o for this process is seen most directly in the $\sim$2 min. delay between maximal ΔT_{ven} and maximal ΔT_{tym} (Fig. 3).

Finally, heat transfer within well-perfused tissues fell to a new minimum, and the T_{art} to T_{ven} gap decreased to a value set by the *fourth equilibration phase* (duration $\sim$23 min) during which the well-perfused tissues equilibrated, as a unit, with a succession of more poorly-perfused compartments, e.g. gut contents, fat, and other tissues comprising the thermal 'periphery' [12]. These processes could be consolidated into a single exponential term. Due to the long time-scale, heat transfer during phase four was probably partly conductive. Estimates of basal metabolism in the anesthetized, non-shivering dog ($\cong$90 J/kg per minute) indicate also that as much as 0.6°C of warming per 20 min in this model may be due to metabolism.

A.3. Thermal accounting

Heat transfer efficiency: Although machine-GLV allowed 2.3 times the lavage frequency of the manual method, and resulted in a larger $\dot{V}_{lav}$ by a factor of 1.2, the cooling magnitudes and rates for machine-GLV significantly ($P < 0.001$) fell short of those obtained with manual-GLV (Fig. 3). The strategy of increasing lavage frequency ($1/t_c$) and decreasing V_{lav}, in order to arrive at approximately the same lavage rate ($\dot{V}_{lav}$), therefore, significantly decreased the fraction of thermal potential which was transferred from each lavage (= heat transfer efficiency, E_f).

Unexpectedly, when the E_f for each of the 8 GLV-cooled animals of Trials I and II (Table 2) was calculated using (Eq. (2)), the value did not differ ($P = 0.46$) between trials; nor did total heat removed per kg (ΔQ_T), as calculated using (Eq. (3)), differ ($P = 0.14$) between Trials. Both of these quantitative methods were therefore inaccurate for some dogs. Calculation of whole-animal mass-specific heat capacities C_m ($= \Delta Q_T/\Delta T_c$) suggested that the Trial II values of ΔQ_T and E_f principally were inaccurate, since the mean C_m value of 0.70 $\pm$ 0.1 cal/g per °K for Trial I was consistent with the C_m reported in the literature for mice and humans [13], whereas C_m values calculated for Trial II using (Eq. (2)) were unrealistic, being greater than the C_m of water.

$$E_f(\text{method 1}) = 1/n\sum (T_S - T_{inf})/(T_{ven} - T_{inf}), \quad (2)$$

$$\Delta Q_T(\text{method 1}) = V_{inv} C_{vf} \sum T_S - T_{inf}. \quad (3)$$

To independently check the accuracy of Trial I values, we computed ΔQ_T and C_m for three dogs from an earlier study (Tables 1 and 2: dogs A, B, and C) that had been given cold PFC with lavage times sufficiently long to allow the volumes and mixed-temperatures of suction-return liquid to be measured for each lavage. This allowed computation of ΔQ_T and E_f by a more detailed method (Method 2, Eqs. (4) and (5)), which used the extra thermal data (not available for Trials I and II) to more directly estimate lavage heat transfer.

ΔQ_T (method 2)

$$= C_{vf} \sum V_{inf}(T_{ven} - T_{inf}) - V_S(T_{ven} - T_{SM}), \quad (4)$$

$$E_f \text{ (method 2)} = 1/n \sum 1 - \left[\frac{V_S(T_{ven} - - T_{SM})}{V_{inf}(T_v - T_{inf})}\right] \quad (5)$$

When this was done, the mean C_m for dogs A, B, and C was found to be 0.68 ± 0.06 cal/g per °K, consistent with the C_m in Trial I ($P = 0.80$). Method 1 (Eqs. (2) and (3)) required the assumptions that PFC suction volume equaled infusion volume, that suction flows remained constant, and that thermal hysteresis was negligible. These assumptions apparently held true at the larger V_{lav} and t_c values of Trial I, but not for the smaller values of Trial II.

With this information, a new E_f for Trial II was estimated using method 3 (Eq. (6)), which employed an estimate for the heat required for the observed ΔT_e, vs. the total PFC thermal-deficit theoretically available. This estimate required a presumed value of C_m. However, if the mean Trial II C_m was assumed to be the same as that of Trial I, then the true Trial II E_f could be calculated (Eq. (6)) to be 0.40 ± 0.06.

$$E_f \text{ (method 3)} = \frac{C_m \Delta T_e}{C_{vf} V_{luv} \sum T_{ven} - T_{inf}} \quad (6)$$

This value agreed with the rough estimation that since Trial II achieved only 73% of the ΔT_e of Trial I, despite using 1.19 times more total PFC (Table 1), the E_f in Trial II was expected to be about 73%/1.19 = 61% that of Trial I.

Thermal compartment size: Heat removal for each cycle (ΔQ_c) in Trial I was calculated from the individual terms of Eq. (3), and individual-cycle mean cooling-power calculated as $\Delta Q_c/t_c$. The latter parameter was useful since thermal compartments in the dog are relatively isolated at short time scales (Fig. 5), and thus the ratio of cooling-power to cooling rate (Eq. (7)) at a given probe site was expected to give the heat capacity (C_h) of the system of thermal compartments that were in equilibrium with each other, and with the site, at the time of the measurement:

$$C_h \text{ (Comp}N) = \frac{m\,[\Delta Q_c/t_c]}{[dT(t)/dt]} \quad (7)$$

C_h (Comp N), total heat capacity of thermal compartments $N = 2 + 3$, or $N = 2 + 3 + 4$.

Both t_c and dT/dt values were picked at a time t, of interest when compartment system N has not yet equilibrated with slower half-time compartment(s). Thus, in Trial I, near the end of lavage cycle # 1 ($t = 30$ s), the lavage thermal-deficit had equilibrated within thermal Compartments 2 + 3 (PFC/viscera/blood-volume), but had not yet significantly reached Compartments 4 or 5. If the cooling rate of T_{ven} at $t = 30$ s (-1.9 ± 0.8°C/min) was then taken as the cooling rate of the system of PFC/viscera/blood-volume, the C_h for this compartment system could be estimated from (Eq. (7)) as $20 \pm 9\%$ of C_T, the total heat capacity of the animal ($C_T = m\ C_m$). Subtracting the C_h contribution of lung PFC ($= m\ V_{luv}\ C_{vf}$) allowed estimation of the remaining tissue C_h for Compartment 3 as $\sim 19 \pm 9\%$ of C_T. Similarly, the C_h of Compartments 2, 3, and 4 together, was estimated at $t = \sim 140$ s (cycle 4) as $71 \pm 17\%$ of C_T, corresponding to the classical whole-body 'thermal core.' The Compartment 5 C_h was then calculated to be the remainder $(100 - 71\%) = 29 \pm 17\%$ of C_T, corresponding to the classical 'thermal periphery.'

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