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EDITORIAL MATTERS

Several months ago we sent out a call for money to begin work on the protective vault for the ALCOR cephalarium. Approximately 8% of our readers responded with donations to the project. We would like to thank those people who gave funds and constructive comments, and at the same time ask those of you who haven't sent a contribution, Why not? Why haven't you made a very simple investment in your future safety? The vault we have on the drawing board at this time would protect the cephalarium from fire, earthquake, and total structural collapse of the facility in which it is housed. Isn't that kind of security worth ten or twenty dollars? A number of people, some who have contributed several times already, have apologized

for sending "small amounts" of money. Every contribution is important and appreciated. So far we have collected \$861 for the vault. We must have at least twice that sum before we can begin construction. So take out your checkbooks, and help guard your future against catastrophe. --Anna Tyeb

LETTERS TO THE EDITORS

JERRY LEAF RESPONDS
TO THOMAS DONALDSON

This letter is in response to Thomas Donaldson's letter to the editors of Cryonics in the April, 1984 issue. I would like to correct his statement that Mike Darwin and I co-authored the paper on cold agglutinins, i.e., Perfusion: Acute Vascular Obstruction and Cold Agglutinins. I am the only author on the paper, so all of Tom's comments concerning this article should have been directed to me alone.

I am gratified that Tom agrees with me: "Every...patient should undergo total body washout." However, he then forces the analogy, "What bothers me about this statement is that this sense is exactly the same in which[sic] we might say, 'Everyone should be immortal.'" This analogy is neither exact, nor accurate, and therefore is a poor analogy, and of questionable value. The analogy falls short for many reasons, but mostly because I know "exactly" how to achieve a total body washout (TBW), but no one knows "exactly" how to be "immortal." It is possible for me to provide suspension patients, even some of them in remote locations, with TBW, but I cannot provide any suspension patient with immortality. It makes sense to say I should provide TBW, because it is a good thing to do and sometimes it can be done, but it is nonsense to say that I should do something that I cannot. Obviously the word should does not have the same meaning as it is used in the analogy. Therefore, the analogy is nonsense. It is reasonable to assert, however, that preventing agglutinated blockage of cryoprotective perfusion, by accomplishing TBW, will relate positively to tissue viability and patient survivability.

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My Paper

The purpose of the article on cold agglutinins was to inform those who are responsible for suspension patient care about what they could do to avoid cold agglutinins. It was not an article for those who cannot provide this care. Tom suggests that something useful might result if remotely located cryonicists operate at a technologically reduced level, say circa 1970. I find this to be less than our suspension patients need. The "good ole days" of cryonics are over, in that we know an uncontrolled perfusion with salt water/DMSO, or a mortician's plunging one of our patients into liquid nitrogen is not the way we should do things. Curtis Henderson said it all: "Cryonics used to be little more than Guerrilla Theatre, but now there is real capability to do suspensions." This is not to say we can always reach out and provide optimum care for every suspension patient. TBW is a relatively simple procedure and requires nothing complex.

The Need To Mobilize

Tom also said, "Given the condition of cryonics today, patients who are transported by commercial aircraft will have been so transported precisely BECAUSE no facilities were available for total body washout." It is true that someone who dies unexpectedly in a place remote from a facility is likely to be shipped by commercial air carrier without TBW. However, it is not true that everyone who dies in a place remote from a facility cannot be provided TBW. With our present capabilities, i.e., "given the condition of cryonics today," Cryovita Laboratories has provided Remote Standby equipment and supplies to go to remote places and do TBW. Today's paramedic services are so effective that most people will make it to a hospital and survive the first 24 hours. The key to using this time is communication with our facility so we can respond. One of the keys to improved remote patient coverage is rapid communication so that we can provide rapid response. An emergency team responding to a call to go to a remote location consists of two trained personnel and four boxes, all ready to fly. Have pump, will travel.

Further, TBW is not some arcane craft which can only be practiced by those blessed by the gods. It is a skill that can be learned and practiced by someone willing to make the effort.

TBW and ECMO

In contrast to remote TBW, cryoprotective perfusion and cooling to solid state hypothermia, at a remote location, is an order of magnitude more complex, for whole bodies. It is possible to do TBW under conditions much less likely to provide a complete suspension. Tom's presumption that, if TBW capability was available then patients could be perfused and frozen, is not necessarily true. It is likely that TBW could be done, even though cryoprotective perfusion and freezing were not possible. Further, perfusion and LN2 cooling are two different problems.

Tom commented that, "Your other transport protocol, of course, is likely to cost at least \$50,000, again presenting a severe problem of a different kind." In fact, this statement does not apply to most suspension members in remote locations. Most suspension members live in the continental United States, with its enormous network of commercial transport. A transcontinental transport, with ECMO, would cost about \$11,000. It may cost Tom \$50,000 to get an air ambulance transport from Australia to California, but then most cryonicists do not live in Canberra. Therefore, ECMO transport is relevant to the "general

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problem," even if it is not relevant to Tom's specific problem.

Future Membership

Tom's concern for future memberships is one that has always concerned cryonicists. However, since his situation has not prevented his continued interest, I offer him as an argument against his expressed concern. Australian cryonicists are the most remotely located in the world, until such time as they develop the skills necessary for independent capability. Furthermore, it should be encouraging to those in remote places that we are developing greater capacity to respond to emergencies of this kind. I have changed nothing by indicating the fact that more response capability is needed for remote members. This has always been the case. I have only committed the indiscretion of speaking the words, "The Emperor has no clothes."

Coming From California

Tom says he doesn't know where I'm "coming from." I will be happy to tell him where I'm coming from: 1. My first concern is for actual existing suspension members. 2. All of my efforts shall be directed to improving the chances of preserving the biological viability and, therefore, the identity and personal survival of suspension members who now exist. 3. I will seek to adopt current high technology to solve the problems we have, rather than resurrect an old technology that has already proved inadequate to our purposes. (I will not try to go to Alpha Centauri in a wagon train just because the space shuttle isn't the perfect vehicle for my ultimate goal). 4. I will never sacrifice a good that I can do today for an existing suspension member, or make unnecessary compromises, for some hypothetical perception by an imaginary suspension member of the future. 5. I will continue to improve the capabilities we have to respond on behalf of our remote suspension members. 6. I will inform suspension members, wherever they are, if there are problems with our ability to do an optimum suspension for them. 7. I will never fall into the deadly trap that befell Robert Nelson and others, by thinking the road to salvation is in future membership. 8. I will never understate my limitations today so that the public will falsely believe I can save them. 9. I will never forget that I must take responsibility for myself. 10. I will do what I can to present an image of hope, because it is the only one we now have, in the context of a full informed consent. This is where I'm coming from.

ALCOR Members

Tom also made a curious statement that might be misinterpreted, when he stated: "Furthermore I know very well that some important members of ALCOR also do not live in Orange County, such as the Chamberlains and a prominent cryobiologist whom I shall not name." As a member of the Board of Directors of ALCOR, I should inform Tom that every member of ALCOR is "important."

Immigration or Exportation

Regarding Mike's article, What You Can Do, I was not involved in writing it, did not review it, and had no prior knowledge of its content. I would agree with Mike that it would be nice to have more cryonicists in Southern California, and it would be easier to serve their suspension needs if they were here. However, I agree with Tom's conclusion that only a small proportion of people interested in cryonics will take up residence in California. I had hoped that

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more cryonics facilities would come into existence and that existing ones would become better prepared. I now have diminished hopes about new facilities. Cryovita has shipped equipment and/or supplies to every existing facility, the least to Michigan and the most to Florida. Mike is right, it is easier to bring a suspension member to a facility created and staffed by skilled people than to take a facility to a suspension member. Remote Standby and ECMO transport are technological responses to the remoteness of many suspension members from existing facilities.

The Phoenix

In a real sense, every suspension member has only one facility, the one his cryonics organization deals with. Even if most cryonicists were concentrated in Orange County, I do not believe the destruction of their

facility would be the end of cryonics. If the people survive, it may not even be the end of the facility, except in a temporary sense. Many organizations have been "wiped out" in the history of cryonics, but key people have moved on, and we now have a greater suspension membership than ever before. Remember the Phoenix, it is a common symbol in the cryonics movement, and cryonicists have earned the right to display that symbol.

Embalming Pumps

I have never used an embalming pump on a suspension patient, but I have used them to pre-filter perfusate. In any case, it is true that any pump is better than a syringe, and I would not fault anyone who used available equipment that could accomplish their purposes. Tom's general theme, concerning equipment, has been discussed many times before, in California. If Tom is proposing that information should be made available about such equipment, then his problem is solved. ALCOR has an extensive historical archive of cryonics literature. These archives contain all the information he will ever want on the use of embalming pumps, embalming cannulas, embalming instruments and embalming techniques. Mike Darwin also has a considerable amount of knowledge of modern mortuary practice. Which may be the reason that over his career in cryonics, he has tended to acquire medical equipment to the almost complete exclusion of mortuary gear.

The Prison Camp

Tom's "prison camp" analogy is perhaps too restrictive. An actual prison camp would have less useful equipment than our average California junk yard. I'd rather send in a Special Forces unit to rescue him at Camp Canberra, but the choice may be out of our hands. It is asking a lot to expect people to do technical things they've only read about. As a matter of fact, Mike tells me that the readers of CRYONICS do not like my technical articles. I have to doubt that it would be of much use to fill the world with substandard equipment and expect it to be used by people who do not even like to read technical material. It has been my observation that people do not want to participate in biological salvage operations. In this sense, they do not want, or feel competent, to save themselves. They want to be saved by their cryonics society, and that is why they became members. Is it actually any wonder our members have this attitude? After all, hasn't all the cryonics literature ever handed out by cryonics societies attempted to convince them that the society could provide cryonic services. What we need to do is make it true.

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The Right Stuff

Tom says, "...the only effective way ANY group can grow is by starting with primitive facilities and working up to the more advanced." It is true that isolated groups of cryonicists can put together modest facilities, if they have the expertise. However, there is absolutely no reason to recapitulate the history of cryonics by starting with embalming pumps. Good, used roller pumps cost less than new embalming pumps. If you want to build a field grade M*A*S*H* unit, at least fill it with the right stuff, it will be less expensive both in the short run and the long run. Regardless of the quality and quantity of equipment and supplies, there is no substitute for preparedness. If you wait until someone dies, before you start looking for equipment and supplies, you are finished before you start, and so is your patient.

Reality

If you find yourself faced with a patient, "...lying in the morgue at 2 degrees"; if there is "only one person available, and that person is untrained, when the patient deanimates;" if you wait until someone dies and then start looking for table salt and embalming pumps; then you weren't prepared for an emergency. If you allow these things to come about, you'll do exactly what everyone else has done in those situations, you'll pack your patients in ice and ship them by commercial air carrier to a facility where there is at least someone who knows something, and is prepared.

The General Problem

Neither medical services nor cryonic services can achieve parity between patients close to facilities and those remote from them. Medical facilities have responded to the needs of distant patients with Medstar helicopters and air ambulances. We have responded with Remote Standby and air ambulances. These are both costly answers to chronic problems involving life and death issues. The general problems of remote services will not be affected by increasing memberships unless the result is an increased number of facilities located closer to those being served.

C.P.R.

Communications. Preparedness. Response. These are the areas that existing cryonics organizations can try to improve. The principles are the same, whether you're talking about three people living in the Alaskan wilderness, or Cryovita Laboratories and ALCOR. In terms of techniques, what IS good enough? We are doing research to answer this question about transport. However complex or simple the procedures become, C.P.R. will always be required. The vital question is, what have you done now so that you can do something when your neighbor deanimates? The question is not, what can I do if I have no capabilities? The question is, what level of capability will I bring to a patient, perfusion or a phone call?

Jerry Leaf
Cryovita Laboratories

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YOU HAVE A DUTY TO DIE

For those who have been fortunate enough to read William McNeill's classic treatment of the impact of disease on human history *Plagues and Peoples* it will come as no surprise to learn that one of the major reasons Christianity spread was because Christians were willing to stand by and take care of their brethren dying of the plague. The pagans would heartlessly abandon their loved ones who were stricken with the plague for fear of contracting the disease themselves. Only the Christians remained in the disease ravaged cities and tended their loved ones more mindful of the bonds of love, community, and individual worth than the burden or the risk of illness.

Recently I have had the opportunity to participate in the care of an older cryonicist who finds himself trapped in a world where he is

considered nothing more than a useless piece of machinery slated for oblivion. Many of those who are caring for him express amazement at our concern for him and the importance with which we regard his continued survival. They are puzzled and incredulous at our unwillingness to abandon one of our own just because he is ill and "old." Our optimism catches everyone off guard. What we are trying to do, what we can see the future holds, is simply beyond the ability of most of the staff who are caring for him to understand.

It should thus come as no surprise that Colorado Governor Richard Lamm has decided that old people "have a duty to die and get out of the way." In a speech given before the Colorado Health Lawyers Association on March 28th the 48-year-old Lamm said: "the elderly have a duty to die and get out of the way. Let the other society, our kids, build a reasonable life." Lamm decried advances in medical technology saying: "We are really approaching a time of almost technological immortality, when the machine and the tubes and the special drugs and the heart pacemakers...literally force life on us."

Lamm believes that extending the lifespan of the elderly is ruining the nation's economic health and creating a world of debt which will be passed down to the younger generation. In the past, Lamm has spoken out against education for the mentally retarded because "after four or five years all they can do is roll over."

On March 28th Lamm addressed a senior citizens' group at Denver's First Baptist Church and urged them to reject machines and wonder drugs likening seniors who took such action to "leaves falling off a tree and forming the humus for other plants to grow up."

It is and will continue to be our great strength as cryonicists that we do not consider each other as leaves: interchangeable and expendable. Because we are willing to acknowledge the worth of ourselves and others as irreplaceable individuals worthy of salvation we have a tremendous advantage. Everyone knows deep down inside that they are more than just a cog in a machine. And if they do not know that they at least want desperately to come to believe it. Certainly, we as cryonicists know that we count as more than fertilizer for people we don't even know--and will never know if we die and vanish forever.

While the world may not know it yet, we have a powerful message. It is, oddly enough, a variant of the same message of early Christianity: NO LEAF OR SPARROW SHALL FALL...WE COUNT AS INDIVIDUALS! This is a message the world is

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hungry to hear. It is a message that gives hope and affirms humanity. It is a message that we can give only by example and its meaning will take time to be understood.

Most of us cryonicists know full well that the fight has just begun. We know full well that the horrors of our now primitive medical capabilities are only the result of treating symptoms rather than causes. Most importantly, we understand clearly that each of us has the potential for rebirth to a life of indefinite growth and flexibility. Because we see the potential of the future to give life to the old and even the "dead" we will not surrender and we will never give up.

Unlike Governor Lamm whose callousness is exceeded only by his stupidity we have the historical advantage that foresight, reason, love and determination bestow. With luck, we may just have the last laugh as well.

WHAT'S WRONG WITH THIS PICTURE?

*** TYPIST'S NOTE: IN THIS SPACE WAS A PICTURE OF AN MEDICAL EMERGENCY BRACELET. ***

It's missing an arm. Which means that somewhere an ALCOR General Member or a BACS Suspension Member is walking around unprotected. Should an emergency occur and the member be unable to communicate, THERE WILL BE NO WAY for anyone to know WHO to call

Think about it. If your ALCOR or BACS bracelet is out of your picture, now's the time to do something about it. Find it! Wear it!

If you are an ALCOR General Member and need to order a replacement or extra bracelet or necktag, simply send us a check or money order for \$8.00 and specify whether you want a bracelet or a necktag.

"Even if the doctor does not give you a year, even if he hesitates about a month, make one brave push and see what can be accomplished in a week."

-- Robert Louis Stevenson

"To travel hopefully is better than to arrive, and the true success is to labor."

-- Robert Louis Stevenson

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NOW, A LITTLE HUMOR

*** TYPIST'S NOTE: BRACKETING THIS PAGE WAS THE ORIGINAL PRINTED TEXT OF THE TABLOID STORY MENTIONED IN THIS ARTICLE ("MAN FROZEN 20 YEARS IS BROUGHT BACK TO LIFE BY SHOCKED DOCTORS" BY KAREN HEALY). ***

Some years ago, Curtis Henderson (former President of the now inactive Cryonics Society of New York) was sitting in a bar less than a mile from the old Cryo-Span facility on Long Island quietly sipping a beer and relaxing. He had come from the tiny industrial bay where he was struggling to maintain just two patients in liquid nitrogen. The television was on, and blaring out the evening news. One of the things mentioned was cryonics.

Two of the patrons in the bar sitting a stool or two away began to talk about cryonics. One man said: "Yeah, you know they got that all worked out. You just have yourself frozen when you die, and wait till they figure out a cure. Then bam! They thaw you out and you're good as new."

"That's bullshit!" the other man said. "You've been watching too much Star Trek."

"No, for real!" the first guy replied. "I saw it on television. Why, they've even

got a place like that right here on Long Island. They've got thousands of corpses stacked up in gleaming white cylinders with technicians keeping a constant watch just waiting for the day when they can revive 'em."

"No kidding," the second guy replied.

"Yep, the government's spending millions on it...I saw it on TV."

Curtis Henderson finished his beer and walked out of the bar without saying a word. He was well aware that it would have been futile to try and inject anything as hard to believe as reality into the two men's media warped image of the world.

The article which appeared in the March 27th issue of the tabloid "Weekly World News" entitled "Man Frozen 20 Years Brought Back to Life by Shocked

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Doctors" will act to generate similar misconceptions in the minds of the naive. For those with some sophistication the article will merely harden disbelief and incredulity about cryonics. For those who are naive the article will form the background for fuzzy stories of success and capability far beyond what reality has to offer. For almost everyone, it will serve to associate our efforts with the likes of faith healing, astrology, celebrity diets, and the other garbage that tabloids feed on.

About all we have to say about the article is: "don't rush out to buy your plane ticket for East Germany yet, just take it for what it's worth: a good laugh.

ALCOR MAY - AUGUST 1984 MEETING CALENDAR

ALCOR meetings are usually held on the first Sunday of the month. Guests are welcome. Unless otherwise noted, meeting start at 1:00 PM.

*** NOTE ***

Since the Tahoe Life Extension Festival is at the end of May, there will be *** NO JUNE MEETING ***

The JULY meeting will be at the home of:

(SUNDAY, 8 JULY 1984) Hugh Hixon
NOTE: **SECOND SUNDAY** 289 Cerritos Avenue
 Long Beach, CA
 Tel: (213) 436-6471

DIRECTIONS: Take the Long Beach Freeway (Hwy 7) south into Long Beach. Take the Broadway offramp and follow Broadway to Alamitos, where Broadway becomes a two-way street. Continue on Broadway two blocks to Cerritos and turn left (north). Go up two blocks to Third Street. 289 is on the southwest corner of Third and Cerritos.

The AUGUST meeting will be at the home of:

(SUNDAY, 5 August 1984) Marce Johnson
 8081 Yorktown Ave.
 Huntington Beach, CA
 Tel: (714) 962-7898

DIRECTIONS: Take Interstate 405 (San Diego Freeway) to Beach Blvd. (Hwy 39) in Huntington Beach. Go south on Beach Blvd. approximately 4-5 miles to Yorktown Ave. Turn left (east) on Yorktown. 8081 is less than one block east, on the north side of the street.

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HEMODIALYZERS AS EXPERIMENTAL HOLLOW FIBER OXYGENATORS
FOR BIOLOGICAL RESEARCH: A PRELIMINARY REPORT

by Jerry Leaf, Mike Federowicz and Hugh Hixon
Cryovita Laboratories, Inc., Fullerton, California

Introduction

The cost of biological research is a limiting factor in both the quantity and the quality of research that can be done. Any innovation that has a wide application and can significantly lessen the cost of experiments will improve the quantity and quality of research performed. This preliminary report describes what we believe to be such an innovation.

A method of oxygenating asanguineous and blood-containing perfusates is a constant need in organ preservation research. Denaturation and hemolysis can result if gases are bubbled through perfusates containing protein or cellular components (1); therefore membrane oxygenators are most desirable, of the several types of oxygenators available.

Current membrane oxygenators, such as the Kolobow (2) or Capiox (3), are intended for the clinical market and thus are costly (\$245 to \$306 per unit) and designed for a single use. Attempts to reuse these clinical oxygenators in our laboratory have met with little success owing to difficulties in cleaning, adequate removal of sterilant, and early failure (often after one or two reuses) due to loss of membrane integrity. In the past ten years, however, hemodialysis as a treatment for kidney failure has resulted in an extremely competitive market for hemodialyzers of low cost, formidable efficiency, and a similar design to clinical hollow fiber oxygenators. Our experiments with currently

Figure 1

*** TYPIST'S NOTE: ***
*** ORIGINALLY THIS SPACE
CONTAINED A DIAGRAM OF A
"HOLLOW FIBER KIDNEY"
(CAPILLARY FLOW DIALYZER);

available hollow fiber dialyzers have demonstrated their adaptability for use as membrane oxygenators. These units cost an average of \$37 when purchased in small quantities, and in clinical practice for dialysis are often reused as many as 12 times. This limit is imposed by a clinical setting where it is desirable to minimize exposure to anticoagulant during exposure to whole blood for the duration of the 3 to 4 hour treatment. In an experimental situation using asanguineous perfusate, loss of fibers in the bundle due to clotting will not be a relevant limitation. This implies that in asanguineous use, hollow fiber dialyzers may have a much larger number

of reuses. Limitations on continued reuse for experimental work will probably bear little relation to those experienced with these devices in hemodialysis. Even assuming that the clinical limit of reuse applies, the cost per use of hollow fiber dialyzer as perfusate oxygenator is still only \$3.06 to \$4.59 per experiment.

A typical hollow fiber dialyzer (Figure 1) consists of a rigid outer shell of polycarbonate plastic and a bundle of approximately 10,000 hollow fibers (this varies

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with the surface area of the device) of cuprammonium-process regenerated cellulose. The ends of the bundle are potted into the polycarbonate housing at each end with a urethane resin. In normal use, blood is passed through the hollow fiber bundle and dialysate is passed through the jacket (outside the fibers), countercurrent to the direction of blood flow. When oxygen is substituted for the dialysate, the dialyzer can function as an oxygenator.

Most dialyzers of one square meter exchange surface are rated at 200-300cc/min flow through the the fiber bundle. However, this stated limitation is primarily due to peculiarities of the hemodialysis system. An increase in flow rate of at least an order of magnitude over the rated flow for dialysis can be achieved in use as an oxygenator by reversing the perfusate and gas pathways, i.e., by passing perfusate through the dialysate pathway and gas through the hollow fibers. Both of these techniques will provide good gas exchange.

The priming volume of a typical dialyzer is usually less than a comparable-sized Kolobow membrane oxygenator (Table 1). Adequate oxygenation can be achieved with a perfusate to gas flow ratio of one to one. Both the Kolobow oxygenator and the hollow fiber dialyzer require an external heat exchanger to control temperature. A comparison of several oxygenators and dialyzers is given in Table 1.

Table 1 Comparison of Oxygenator and Dialyzer Specifications

	Oxygenators		Dialyzers		
	Kolobow 0.8sq m	Capiox II CXMP16H	Travenol 12.11	Erika HPF200	C-Dak 1.8
Surface area (Sq. meters)	0.8	1.6	0.8	1.0	1.8
Prime volume (cc.)	100	120	61	60	135
Max flow rate (rated cc/min)	1200	2000	250	250	250
Ultrafiltration coefficient			3.2	3.6	2.2
Cost/unit (dollars)	\$245	\$306	\$36.75		
Number of uses (clin pract)	1	1	8-12	8-12	8-12
Cost/use	\$245	\$306	\$3.06		

Both oxygenators are pediatric size. The gas flow rate for the oxygenators is equal to the blood flow rate in normal usage. The Kolobow is of spiral-wound design. The Capiiox II is a hollow fiber oxygenator. Rated dialyzer flow rates do not reflect real dialyzer flow capacity, since in clinical applications intake into the dialysis system is through a 16ga needle.

Hollow fiber dialyzers do not have hydrophobic membranes; therefore, ultrafiltration (UF) (removal of water from perfusates) can occur. The quantity of ultrafiltrate will be primarily determined by the pressure difference across the walls of the hollow fibers and the total surface area of the fiber bundle. However, UF can be completely eliminated by adjusting the gas pressure to equal the hydrostatic pressure of the perfusate inside the fibers. This can be easily accomplished by placing a screw clamp on the gas outlet line and restricting gas outflow until the pressures are equal. Aneroid manometers are placed on the

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gas and fluid pathways to provide a simple means of monitoring these pressures. The rate of ultrafiltration even without regulating the transmembrane pressure is usually modest enough that the ultrafiltrate can be returned to the perfusate reservoir without significantly influencing arterial perfusate composition.

The ability of hollow fiber dialyzers to ultrafilter water and accompanying solutes below a molecular weight of 1,000 daltons may in some situations be an advantage. The ability of these devices to retain colloids and formed elements of the blood allows for dynamic variation of colloid osmotic pressure (COP) and hematocrit without resort to addition of colloids or packed cells and without affecting the cryoprotective or electrolyte concentration of the perfusate. This feature of the devices has allowed us to achieve hemoconcentration of blood/perfusate mixtures following partial bypass in both the dog (4) and the cat and thus avoid the need for transfusion following termination of extracorporeal support. Ability to control COP independent of cryoprotective agent (CPA) or electrolyte concentration has obvious advantages in the management of developing edema in both the intact animal and the isolated organ.

The rate of ultrafiltration for a given situation is the product of the average pressure across the walls of the fibers and the ultrafiltration coefficient (KUF). KUF is expressed as ml of ultrafiltrate per hour per mm Hg of transmembrane pressure (TMP) (i.e., pressure differential across the membrane)(5). Ultrafiltration coefficients vary as a function of dialyzer surface area and composition of membrane material and are specific to the model of dialyzer. The KUF is simple to evaluate and provides a method of monitoring the condition of individual dialyzers. The rated KUF's for the dialyzers used in this study are shown in Table 1.

By restricting gas outflow it is possible to lower the transmembrane pressure and control very precisely the rate of ultrafiltration. Alternatively, restricting fluid outflow can be used to increase the hydrostatic pressure within the fibers increasing the rate of ultrafiltration. The relationship between ultrafiltration rate and transmembrane pressure is:

$$V \text{ (cc)/t (hours)} = \text{KUF} \times \text{TMP (mm Hg)}$$

where

$$\text{TMP} = (\text{average pressure inside fibers}) - (\text{av press outside fibers})$$

and the pressure averages are the average of the inlet and outlet pressures on each side of the system.

Two other uses for hollow fiber dialyzers which we have not investigated in detail are as heat exchangers and for adding and removing low molecular weight cryoprotective agents. Where expensive formed blood elements, colloids, or plasma proteins are being used in perfusion, their use may be minimized by restricting them to the organ perfusion circuit and moving gases, small molecules, and heat into and out of the circuit across the membrane more or less simultaneously.

Tests of Gas Transfer

Hollow fiber dialyzers (6) were tested for their ability to transfer oxygen and carbon dioxide with the test circuit shown in Figure 2. The dialyzer was an Erika HPF200. A Travenol Miniprime disposable heat exchanger (7) was placed in front of the dialyzer to adjust perfusate temperature before it entered the fiber bundle, in order to eliminate the effect of temperature on gas solubility.

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A Sarns heater/cooler was used as the temperature controller. Distilled water was used as a test perfusate and was run countercurrent to the gas flow.

Figure 2

*** TYPIST'S NOTE: ORIGINALLY THIS SPACE CONTAINED A SCHEMATIC DIAGRAM OF THE "OXYGENATOR TEST CIRCUIT." ***

Efficacy of oxygen transfer (9) was evaluated at 8, 17, and 38 degrees centigrade at perfusate flow rates of 50cc/min., 150cc/min., and 250cc/min. Pure oxygen was passed through the dialysate pathway at 1 liter/min for all conditions of perfusate flow and temperature. Test samples were drawn at the inlet and at 6 inches beyond the outlet port of the dialyzer. The perfusate temperature differential from inlet to outlet was less than 1 degree centigrade. Gas transfers were calculated and plotted against perfusate flow rate at each temperature (Figure 3).

Figure 3

*** TYPIST'S NOTE: ORIGINALLY THIS SPACE CONTAINED AN "OXYGEN TRANSFER" GRAPH, OF OXYGEN TRANSFER VOLUME % COMPARED TO PERFUSATE FLOW IN CC/MIN, AT 8°C, 17°C, AND 38°C.***

(14)

Carbon dioxide transfers (9) were evaluated at 17 and 38 degrees centigrade for perfusate flow rates of 50cc/min., 150cc/min. and 300cc/min. The circuit employed was the same as for the oxygen transfer test described above. A 5% carbon dioxide/95% oxygen gas mixture was delivered at a constant flow rate of 1 liter/min. for all conditions of perfusate flow and temperature. Temperature differential from inlet to outlet varied less than 1 degree centigrade for all conditions. Gas transfers were calculated and plotted against perfusate flow rate at each temperature (Figure 4).

Figure 4

*** ORIGINALLY THIS SPACE CONTAINED A "CO2 TRANSFER" GRAPH, OF CO2 TRANSFER VOLUME % COMPARED TO PERFUSATE FLOW CC/MIN, AT BOTH 17°C AND 38°C. ***

Table 2 Comparison Between Normal and Reversed Transfer Rates

Perfusate flow (cc/min)	Normal flow direction		Reversed flow direction	
	Temp (deg C)	Oxygen (vol %)	Temp (deg C)	Oxygen (vol %)
50	9	34.3	10	34.3
100	10	35.8	11	27.6
200	13	26.4	12	20.5

Tests were run on an Erika HPF200 dialyzer. Oxygen flow rate was held equal to perfusate flow rate.

As noted above, clinical considerations restrict the maximum flow of blood to approximately 250cc/min, and the ports of the dialyzer are engineered accordingly. Reversing the normal flow paths and allowing the gas to flow through the 3/16" diameter blood inlet/outlet ports, and the perfusate to flow through the 3/8" ports of the dialysate path would allow for improved flow rate of perfusate. A third test was performed using the same circuit with the paths in normal configuration and then reversed in this manner. As can be seen in Table 2, there is little difference in the efficiency of gas exchange

(15)

over the flow rates and temperatures tested. In actual laboratory practice using a Cordis Dow 2.5 square meter hollow fiber dialyzer in reversed configuration, we have run blood flows of 4 liters/min., approximately sixteen times the rated flow rate of the dialyzer, without significant denaturation or damage to the formed elements of the blood (4).

Blood Oxygenation

Table 3 Oxygenation of Blood by Dialyzer

Blood flow rate (cc/min)	Temp (deg C)	Oxygen (vol%)	
		Venous	Arterial
50	37	8.78	15.09
100	37	8.14	14.88
200	37	7.24	11.36

Tests were run with Travenol 12.11 dialyzer. Oxygen rate was held to 100 cc/min.

The test dialyzer, a Travenol 12.11 (Table 1), was connected in parallel with the oxygenator of a heart-lung machine supporting a dog on total cardiopulmonary bypass. Venous blood was pumped through the dialyzer fibers at three flow rates, 50cc/min., 100cc/min., and 200cc/min. Oxygen flow was held constant at 100cc/min., and blood temperature at 37 degrees centigrade. Blood samples were taken from the inlet and outlet ports. Total oxygen content determinations were made, using the Severinghaus technique (8).

Table 3 shows the inlet (venous) versus outlet (arterial) oxygen content, in volume percent at each of the three flows. It is evident that an

experimental animal such as the cat (resting cardiac output of approximately 280cc/min. at normothermia for a 4kg. animal) could easily be supported on total cardiopulmonary bypass with a 0.8 square meter hollow fiber dialyzer acting as an oxygenator. The fall-off in blood oxygenation at the higher flow rates is at least partly due to the single gas flow rate used, instead of the 1:1 gas-to-perfusate ratio that is usually employed in oxygenators.

Cryoprotective Perfusion

The perfusion circuit employed for these experiments is shown in Figure 5. A Shiley BCD heat exchanger (7) was used to control temperature and act as a bubble trap. The heat exchanger was positioned on the output side of the oxygenator. A Pall 40-micron filter was placed in the arterial line to protect against particulate emboli. A Cordis Dow C-Dak 1.8 square meter dialyzer (9) was used as an oxygenator. The roller pump employed in this circuit was a Sarns Model 1500. A Gould-Statham Cannulating Flow Sensor and SP/2202 flow meter were used to monitor perfusate flow. Additionally, the Sarns pump was calibrated to provide a check of flowmeter accuracy.

A cat was anesthetized with 30 mg/kg nembutal and ventilated on a Physiograph pressure regulated respirator until completion of total body washout. An arterial perfusion cannula was placed in the aortic root through a purse-string suture and a venous cannula in the apex of the right atrium. An arterial pressure line was placed in the left femoral artery. A Collins-type base perfusate, KCl 0.28M, K₂HPO₄ 0.0054M, NaHCO₃ 0.01M, disodium glycerophosphate 0.027M, MgCl₂ 0.0043M, CaCl₂ 0.001M, dextrose 0.011M, mannitol 0.118M, Dextran-40 50g/liter, was used for total body washout. Perfusate temperature was maintained between 10 and 12 degrees centigrade. Mean arterial pressure was held at 50 mm Hg. Perfusate containing 6.85M glycerol was

*** TYPIST'S NOTE: THE NEXT FOUR PAGES CONTAINED PHOTOGRAPHS. THE FOLLOWING TEXT APPEARED WITH THE PHOTOS. ***

(PAGE 1)

THESE PHOTOGRAPHS ACCOMPANY THE ARTICLE "SIMPLE CRYOGENIC TECHNIQUES -- II" WHICH BEGINS ON PAGE 26.

Photo G (Left) shows the lid-mounted sensors. The wand coming out of the insulating plug is the level sensor. The long bulb in the foam plug next to the rim is the high-flow-rate sensor.

Photo H (right) shows the Napco dialer alarm system that is connected to the telephone lines.

(PAGE 2)

Photo (Above) shows the alarm switch boxes on the top of the dewar lid. The chain and lock are for security.

(PAGE 3)

Photo I (Right) shows the baseboard of the rocker stretcher system. A rod hooks into the hole in the board for placing and removing the base. The black and white paint design allows orientation of the stretchers when the base is under LN₂.

Photo J (Left) shows the stretchers set in the baseplate with one tilted as it might be to allow entry of the other stretcher into the dewar.

Photo K (Above) shows a patient bag being lowered into the outer container for neuropreservation.

Photo L (Rt. above) shows the bag, with an attached ID tag in the neuropreservation container.

Photo M (Right) shows the container with the lid on, ready to be wired shut.

(16)

Figure 5

*** TYPIST'S NOTE: ORIGINALLY THIS SPACE CONTAINED A SCHEMATIC DIAGRAM OF AN "ORGAN PERFUSION CIRCUIT." ***

Table 4 Gas Exchange with Glycerol Perfusate

Time(min)	Oxygen		CO2	
	Venous	Arterial	Ven.	Art.
10	198	596	23.8	15.4
30	286	607	23.4	15.3
50	281	599	23.6	15.3
70	250	591	23.2	21.0
100	224	547	23.6	15.1

added to the circuit reservoir at a constant rate over two hours until a terminal concentration of 3.2M glycerol was achieved.

Arterial and venous samples were drawn from their respective cannulas and oxygen and carbon dioxide determinations were made on a Radiometer BMS-3 blood gas system. Table 4 shows oxygenator performance in terms of perfusate arterial and venous oxygen and carbon dioxide concentrations. In this model, metabolic acidosis can only be controlled by providing adequate oxygen delivery. The pH of the arterial perfusate averaged 7.45 over the two hour perfusion, with a range of 7.36 to 7.65 (11). Oxygen to perfusate flow was kept at a 1:1 ratio.

Values are gas pressures in mm Hg. The dialyzer was a C-Dak 1.8 sq. meter unit. Glycerol concentration was from 0M to 3.2M, over a 120 min. period.

(17)

Reuse

Due to the increasing need for cost containment, systems for reuse of hollow fiber dialyzers are undergoing rapid evolution. Currently, many institutions employ formaldehyde as the sterilant for clinical reuse. This is the only agent with which we have had experience and most of the discussion which follows will be based on the use of formaldehyde.

Following the the conclusion of an experiment, blood and/or perfusate is thoroughly rinsed from the dialyzer using filtered tap water. Care is

taken not to exceed a blood path pressure of 500 mm Hg in order to protect the fibers against rupture. When the fiber bundle (or alternatively the fiber bundle exterior, if the dialysate path has been used for blood/perfusate) has been thoroughly cleared of visible blood or perfusate, the unit is further rinsed with 4-5 liters of deionized-reverse osmosis (DI/RO) treated water. Following the DI/RO rinse both the fluid and gas paths are filled with a 3% formaldehyde solution and all ports to the device are capped. If the entire perfusion circuit is to be retained, it is similarly rinsed and treated with formaldehyde solution. In our experience dialyzers and entire perfusion circuits treated in this manner have a more or less indefinite shelf life. After each reuse the device is evaluated for accumulation of proteinaceous debris or clots at both headers and in the fiber bundle as well. Functional evaluations of the device during use (with respect to gas transfer and/or ultrafiltration) are also undertaken if reuse is to extend beyond three or four times. In our experience even extensive reuse is not associated with any change in gas exchange performance.

Many complications and problems with dialyzer reuse have been reported in the clinical literature (12). However, most of these problems seem secondary to retention of blood in the device as a consequence of clotting. Immunological effects, in particular the formation of an N-like antibody in response to exposure of patients to their own fixed proteins (which coat the fibers and remain on the membrane following sterilization) has also been reported (13) (14) (15). Additionally, there have been changes in membrane performance (related to clearance of waste products and the coefficient of ultrafiltration) with prolonged reuse of dialyzers in a clinical setting (16).

Dialyzers are prepared for reuse by first rinsing them with 20 to 30 liters of filtered tap water delivered simultaneously to both fluid and gas paths. Following tap water rinsing, a crude test for the presence of formaldehyde is made by placing 1/2 cc of effluent from the blood path in a test tube and adding 1 Clinitest tablet (17). A positive reaction (any color change other than deep blue) implies the presence of formaldehyde and continued rinsing with tap water is undertaken. Following a negative Clinitest the device is rinsed with 4 to 5 liters of DI/RO water and re-tested for the presence of formaldehyde by the Hantzsch reaction (18) employing Schiff's reagent (Formatest(19)). The Clinitest is not used as the final test for the presence of formaldehyde as it lacks sufficient sensitivity (15). The Clinitest test is useful for preparing the dialyzers for reuse in that its cost per test is a tiny fraction of that of the Formatest.

Because of the many undesirable properties of formaldehyde (i.e., odor, toxicity, carcinogenicity) (20) a new agent for sterilizing dialyzers for reuse has recently been approved by the FDA. Renalin(TM) (21), a stabilized solution of hydrogen peroxide and peracetic acid (peroxyacetic acid), reportedly is not associated with the formation of N-like antibodies and is not as noxious as formaldehyde. We have no experience with this agent. However, initial reports from dialysis centers employing peracetic acid for reuse have been favorable (22).

(18)

Summary-Conclusion

Hollow fiber dialyzers can be used as membrane oxygenators for organ preservation studies. Dialyzers used as experimental oxygenators have been shown to meet more than adequately the flow, oxygen, and carbon dioxide requirements for isolated organ perfusion. Gas transfer data from test circuits and animal perfusions have been presented to demonstrate the

performance of these membrane oxygenators.

We have used hollow fiber dialyzers to supply the oxygen requirements of intact small animals during cryoprotective perfusion, using 1 square meter dialyzers. In the future we will publish data showing that large mammals, such as dogs, can be provided adequate oxygenation using 2.5 square meter Cordis Dow dialyzers. The oxygenation performance range for hollow fiber dialyzers ranges from isolated organs to large mammals.

In selecting dialyzers for use as oxygenators, we have found cost and availability to be the dominant criteria. Even the smallest dialyzers are entirely adequate for animals up to the size of a small dog, or any organ from a human or a smaller animal. Because of manufacturing differences, the available exchange surface and flow rate figures given by manufacturers cannot be used to compare dialyzers except in the crudest way, even as dialyzers.

We summarize the advantages of hollow fiber dialyzers used as oxygenators as follows: 1) They provide membrane oxygenation, resulting in minimal damage to blood components; 2) Their unit cost is much lower; 3) They are reusable a minimum of 8 to 12 times with proper storage techniques. When this is combined with 2), their cost per experiment is extremely low; 4) They have considerably reduced priming volumes; 5) They do not have any special priming requirements; 6) They require less gas than bubble oxygenators to provide adequate oxygenation.

It is hoped that other investigators will find these oxygenators useful in their research and that the resultant reduced cost of experimentation will contribute to an improvement in organ preservation research.

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- 7) Travenol Miniprime Disposable Hypothermic Heat Exchanger for use with oxygenators, 1/4 in. inlet/outlet ports. Manufactured by Artificial Organs Division, Travenol Laboratories, Inc., Deerfield, IL. Shiley BCD (Buckberg-Shiley Blood Cardioplegic Solution Delivery Set). We used the reservoir with the heat exchanger only, and not the tubing normally supplied with these units. Manufactured by Shiley, Inc., Irvine, CA.

(19)

- 8) "Electrode for Blood PO₂ and PCO₂ Determination," J. Severinghaus and A. Bradley, J. Appl. Physiol., 13, 515-20 (1958).
- 9) Gas transfers are defined:
Gas transfer = (ml gas/100 ml perfusate) x (ml perfusate/min)/100
- 10) Cordis Dow C-Dak, 1.8 sq. meter hollow fiber kidney. Manufactured by

Cordis Dow Corporation, Miami, FL.

- 11) pH measurements were made at 37 deg. C in a Radiometer BMS-3 blood gas analyzer. The pH values given in the text are uncorrected for the temperature of the perfusate. Corrected values can be calculated by:
 $\text{pH}(\text{corrected}) = \text{pH}(\text{measured}) \times 0.0147 \times (\text{degrees C below } 37 \text{ degrees C})$
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- 16) "Evaluation of hemodialyzers and dialysis membranes. Report of the Study Group for the Artificial Kidney-Chronic Uremia Program," U.S. Department of Health, Education, and Welfare, Public Health Service, Rep. #77-1294, (1977).
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- 22) "Experience with the Disinfectant Peroxyacetic Acid (PES) for Hemodialyzer Reuse," H. Bauer, H. Brunner, and H.E. Franz, Paper to the American Society for Artificial Internal Organs (ASAI0), April 28-30, 1983, at Toronto, Canada, (Abstracts, Vol 12)

REPORT ON THE 20TH ANNUAL MEETING OF
THE SOCIETY FOR CRYOBIOLOGY -- PART IV

In this fourth and final installment of our coverage of the 1983 meeting of the Society for Cryobiology we continue describing advances in fundamental aspects of cryobiology and conclude with miscellaneous points of interest.

SYMPOSIUM: MEMBRANES AT LOW TEMPERATURES

Membranes have generally been thought to be the primary targets of freezing injury. They are also implicated in "thermal shock", a form of damage seen in some systems after rapid cooling alone (no freezing). Consequently the symposium on membranes at low temperatures was of automatic interest to

cryobiologists. The interest for cryonics was more limited, but certainly

not negligible.

D. Chapman (Royal Free Hospital School of Medicine, London) began the symposium by considering membrane phase transitions, i.e., the freezing of lipid components of membranes. When membranes freeze, "islands" of crystallized material are formed in a "sea" of dissimilar membrane lipid and protein. This may lead to defects in the membrane structure (holes) due to the inability of membrane components to associate closely enough to prevent gaps from forming (packing defects), or perhaps due to disruptive collisions of the frozen islands. The result is an increase in membrane permeability and possible cell damage. Membrane phase transitions can be caused by temperature reduction or by dehydration (or both). Cholesterol helps reduce the consequences of temperature lowering, acting much the way a cryoprotectant acts in an aqueous system. Trehalose can substitute for membrane water and thus prevent dehydration-induced injury.

On the other hand, phase transitions of the kind considered by Chapman may actually be irrelevant to cryonicists, since they will generally occur well above 0 degrees centigrade whereas mammals can survive cooling to 0 degrees centigrade or below.

The second speaker (A. Clarke, British Antarctic Survey, UK) pointed out that although phase transitions in membranes correlate with membrane failure in many cases, many organisms grow well with (and may even require) partially crystallized membranes and that membrane phase transitions do not always result in increased permeability. Furthermore, there seems to be no correlation between freeze-thaw recoveries and the freezing points of membrane lipids, at least in the case of algae.

G.J. Morris (Institute of Terrestrial Ecology, UK) continued on the phase transition theme by speculatively linking it to both thermal shock (of cold shock) and freezing injury. He speculated first that all cells are susceptible to cold shock if the cooling rate is high enough and/or if the temperature is low enough. He then speculated that traditional freezing injury is largely a combination of chilling injury and cold shock, chilling injury accounting for slow-freezing injury and cold shock inducing intracellular freezing (fast-freezing injury), with the optimal cooling rate minimizing both chilling injury and cold shock. (Chilling injury only is found in systems cooled without freezing to sufficiently low temperatures and held there or cooled very slowly. The damage seen in kidneys supercooled to -4 degrees to -7 degrees centigrade is one example of chilling injury). Finally, he proposed a possible link between freezing injury and membrane phase transitions; supposing that chilling injury is due to lipid crystallization in the cells membrane. At low cooling rates or at a constant temperature a few crystals would form and grow to large size. The mismatch between liquid and crystal around these large crystals would be large and at slow cooling rates would have plenty of time to be destructive (favorable conditions for collision of lipid islands would be present).

As the cooling rate is raised the mismatch is reduced (smaller crystals) and time for destructive processes is reduced. But as the cooling rate is raised still further, packing defects become more serious because the membrane consists of a jumble of rigid little lipid crystals whose boundaries do not fit together. Since the cell will be supercooled under conditions of rapid cooling, ice can grow through the packing defects and nucleate the intracellular water. Thus, freezing damage, according to Morris' speculations, is basically a consequence of membrane phase transitions no matter how you look at it.

Morris' theories are radically different from standard theories of freezing injury. They are also, probably, largely incorrect. However, there may be

enough validity to them to offer important new insights and approaches to cryoprotection. If nothing else they should give cryobiologists something to argue over, and that kind of arguing is stimulating and useful in science.

P. Steponkus presented the next paper, which dealt again with his plant protoplast model system (readers may notice the frequency with which Steponkus' name appears in this report. He was associated with 13 papers and presentations at this conference, continuing his tradition of prolific, exemplary science). In this presentation he showed how many different manifestations of injury such as popping during cell re-expansion, loss of osmotic responsiveness, and intracellular nucleation are really the result of the same membrane properties responding to different conditions of stress. He emphasized his concept of the cause of intracellular freezing being not intracellular nucleating agents but rather, growth of ice through a membrane defect caused by excessive dehydration injury. This concept should be particularly helpful in clarifying the thinking of many cryobiologists outside the field of plant freezing. Steponkus also introduced a novel concept in cryobiology, namely, the idea that so-called freezing potentials are important. Freezing potentials arise in very dilute electrolyte solutions because ice actually can trap minute quantities of electrolyte, but favors one charge over another. Consequently, charge separation occurs at an ice-water interface and cells in the vicinity may be damaged. However, Steponkus' data supporting the relevance of this phenomenon to protoplasts seemed unconvincing, and in animal systems the electrolyte concentration should be high enough to short out the freezing potentials, rendering it insignificant.

Joe Wolfe (University of New South Wales, Australia) then followed with an exciting description of the forces which in theory can account for membrane failures of the types found by Steponkus, and which may apply to frozen cells (of more direct interest to cryonicists).

For example, when cells are frozen, cell volume is reduced due to the transmembrane osmotic gradient that is set up, as many cryonicists are aware. But what happens to the cell membrane? Two possibilities have traditionally been proposed. First, the cell may behave like a balloon made of thin paper. As the balloon is deflated, the membranes crumple and folds but does not change area or resist cell shrinkage. This is the Mazur model, used to predict how cells shrink during freezing at different rates. Second, the cell may behave like a balloon made of rubber, the surface area changing according to the surface-to-volume ratio of a shrinking sphere, for instance.

But unlike a rubber balloon, whose resting state is the deflated state, the cell membrane is not under a great deal of stretching force (tension) in the "inflated" condition (normal cell volume). Consequently, as the cell shrinks, there is not simply a relief of stretching but rather the reverse, that is, an increase in pressure caused by the crowding together of membrane components. This increased pressure causes a resistance to cell volume reduction which in turn allows for a permanent transmembrane osmotic pressure difference to be maintained. The cell is damaged as a consequence of increased membrane pressure. This is the Meryman/Williams model of freezing injury (the minimal cell volume hypothesis). In extreme cases no cell volume reduction takes place at all. The cell behaves like a diving bell; the membrane resists shrinkage despite a huge pressure difference between the inside and the outside of the cell.

In considering the known mechanical properties of membranes, Wolfe finds that membranes are simply physically unable to maintain a pressure difference as proposed by Meryman et. al. Any appreciable pressure causes buckling or folding, which relieves the pressure. The cell membrane will therefore tend to remain constant in area during freezing, as supposed by Mazur, unless it loses

material. Plant protoplasts initially show shrinkage without area loss but then area is lost to restore the cells to their native spherical shape (minimal surface area). Upon re-expansion the cells pop due to the reduced area; Wolfe was able to model this popping phenomenon nicely. (Membrane mechanics also formed the basis of discussion in two workshops on the role of thermodynamic modeling in cryobiology. Basically, the workshops seemed devoted to destroying the Meryman/Williams model. One particularly noteworthy event was the participation of a supremely talented and qualified membrane mechanics scientist, Evan Evans, who was not previously familiar with cryobiology but who was able to offer a great deal of constructive comment. Hopefully these workshops' influence will show up in future cryobiological publications and clarify whether the minimal cell volume theory can survive).

"Membranes in Low Temperature Injury" was the title of session 10. Takahashi et. al. reported that hypertonic salt reduced membrane fluidity of human red blood cell membranes and hypertonic sucrose caused a new, immobile phase to form in these membranes. Membrane probes and electron microscopy showed evidence for a membrane phase transition taking place at the same temperature that induces thermal shock during cooling of hypertonic red cells (10 degrees centigrade). In other words, more detailed understanding of hypertonic damage and of the nature of thermal shock is emerging. A second paper by Takahashi reported that thermal shock can be induced without high salt or sucrose concentration by using drugs that affect cell calcium regulation. Whether cryonicists might inadvertently be able to promote thermal shock in suspension patients being cooled in the presence of drugs of this class is somewhat doubtful but worth considering.

The best kind of advance in fundamental cryobiology, of course, is one that improves not only understanding but also freeze-thaw survival. G. Matthes et. al. (East Germany) provided both by studies on free radical damage in cryobiology. They treated atrial (heart muscle) strips with 3.5M dimethyl sulfoxide, cooled them (1 degree centigrade/minute) to -25 degrees centigrade, held them for 30 minutes, and then plunged them into liquid nitrogen. When penicillin was included in the solutions used, survival rates went from 10% to 100%! Penicillin stimulates peroxidase activity. Selenomethionine and vitamin E also raised percent survival and reduced tissue levels of malondialdehyde, a byproduct of lipid peroxidation. The level of selenomethionine used was 10 to the minus 9 molar. The value of these observations is obvious, and it may very well be prudent to include these agents, in nontoxic concentrations, in cryonics perfusates. We certainly hope to see independent verification of these results.

William J. Gordon-Kamm and P. Steponkus presented freeze-fracture data giving more insight into protoplast cryoinjury. Shrunken non-acclimated protoplasts lost blobs of unmodified membrane into the cytoplasm to form vesicles. Shrunken acclimated protoplasts, by contrast, developed projections on the cell surface which were enriched in membrane lipid. (Unlike the cytoplasmic vesicles, the extracellular "tentacles" of extruded lipid can be re-incorporated into the membrane proper during cellular re-expansion, preventing the cells from "popping").

Session 14 was "Nucleation, Crystallization and Vitrification". It began with Sheila Mathias, Felix Franks, and Kay Trafford's study suggesting that red cells may contain intracellular nucleating agents associated with the inner face of the cell membrane. Steponkus and Dowgert then presented data showing that intracellular freezing of protoplasts took place at different temperatures depending on the ionic or non-ionic nature of the extracellular solutes, the presence of proline, the presence of

eutectic freezing and the cooling rate. The data suggested cell nucleation as result of freezing damage rather than to the presence of intracellular nucleating agents.

(23)

Pierre Boutron (author of a book on immortality and cryonics some years ago) presented a mathematical study of the effect of cooling rate on the vitrification behavior of solutions. He was able to account quantitatively for his previously-measured reduction in amount of ice formed as cooling rate increases. This study may be important in the context of models of cell dehydration during cooling because such models assume that the amount of ice formed does not depend on cooling rate. Boutron's results would likely not be relevant in the area of vitrifying whole brains or whole people since relatively low cooling rates would be unavoidable for such large systems.

The session was ended by a presentation by William E. Brower (of Cocks and Brower, being the two metallurgists who introduced three-component phase diagrams into cryobiology). He made the claim that 40% glycerol plus 4.4% sodium chloride could vitrify at a cooling rate of 100 degrees centigrade/minute and that devitrification could be avoided at a heating rate of 50 degrees centigrade/minute. This is doubtful but would be exciting if true. He also reported that at 10 degrees centigrade/minute and with 1% sodium chloride present (1% sodium chloride being approximately isotonic), at least 65% glycerol had to be present to form a glass.

The final session of the meeting concerned low temperature physical chemistry and plant cryobiology. Perhaps the most important paper in this session was also one of the simplest and most straightforward. This was D.E. Pegg's paper describing very simple equations that allow one to calculate phase diagram information for the glycerol-sodium chloride-water system. The equations are as follows:

$$(1) \quad MP = C(-1.6 - 1.27R - 0.25R)$$

$$(2) \quad ET = -21.2 - 4.55R - 0.55R$$

$$(3) \quad ET = -46.5 - 234.4/R$$

MP is the freezing point of a solution of total concentration C (% w/w) and having a weight ratio of glycerol to salt of R. Most carrier solutions used in cryonics or in cryonics research can be assumed to be equivalent to a 1% sodium chloride solution as a reasonable approximation. ET stands for eutectic temperature. Equation 2 is used if R is less than or equal to 7, and equation 3 is used for R greater than 7.

Two papers offered were concerned with pH control at subzero temperatures. The first, by Alan MacKenzie (University of Washington, Seattle) was about phase diagram behavior and pH behavior of Tris buffer. Unfortunately it is difficult to see the relevance of MacKenzie's system since nothing other than Tris was present and since, of course, Tris would be one of the last buffers anyone would select for either cryonics or cryobiological purposes. R.N. Roy's paper on the behavior of "good" buffers such as bicine was more germane. A bicine buffer is 40% dimethyl sulfoxide will change pH by about one pH unit during cooling from 25 degrees centigrade to -15 degrees centigrade (no freezing).

MISCELLANY

Most insects experimentally transferred to the Antarctic from more temperate zones die. However, the chironomide midge fly and the

enchytraeid worm have been surviving nicely for the past 16 years at Signy Island being after being transplanted there by an earlier expedition from either South Georgia or the Falkland Islands. Perhaps freezing isn't so hard to survive after all. (Abstract #23, W. Block et. al., British Antarctic Survey).

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Mary Ann Brock submitted an abstract (from the NIA in Baltimore) showing that lymphocytes from old C57Bl/6 mice showed slightly reduced freeze-thaw survival (60%) compared to freeze-thaw survival of young lymphocytes (80%). They also seemed to show selective losses of some stimulus-response systems. It looks as though Mary is pursuing her dual goals (her primary training is as a gerontologist) with some interesting results. (Abstract 76)

Shades of cryobiologists past returned to life in the film "Low Temperature Biology at Mill Hill", which was shown at the "Conversazione" the first day of the meeting. There again was the crusty Audrey Smith showing us how sperm and red cells survive freezing in her primitive cryomicroscope. Then we were treated to the sight of Christopher Polge artificially inseminating a cow with bull sperm he had recently learned to freeze successfully. But the best part by far was the engrossing footage showing J.E. Lovelock, with the aid of Audrey Smith and R. Andjus, calmly and matter-of-factly explaining and then demonstrating the survival of partly-frozen hamsters. It should be noted that Lovelock's microwave warming system was incredibly sophisticated for its time. This film could easily have been made as a clip for a Hollywood Frankenstein movie, with its players having that just-right look of '40's oldness and laboratory costuming. Somehow and somewhere, a copy of this film must fall into the hands of cryonicists. After all, this is really cryonics history being chronicled, as well as that of cryobiology. The film ended with a very young Michael Ashwood-Smith describing the use of dimethyl sulfoxide for bone marrow freezing. (Abstract 82)

Just how inherently damaging is freezing? One indication is how deeply frozen unprotected cells must be to be killed. Walker carcinoma cells survive unprotected freezing to -30 degrees centigrade and are only killed when frozen to -40 degrees centigrade (Abstract 92). Why do these cells survive such an incredibly severe freeze when others do not? Maybe most cells are only damaged at a few sites of specialization.

How much punishment can a vein take and still function? A lot, it seems. G.S. McIntosh and K.E.F. Hobbs (Abstract 93) froze pigs' portal veins to -100 degrees centigrade not once but twice. They found blood flowing though the veins 6 weeks later, although admittedly the veins did show damage microscopically.

One real surprise was an abstract (#140) submitted from the People's Republic of China, detailing the occurrence of supercooling in a rat's tail. They reported that if the rat's tail is placed in a cooling bath in the winter, the tail supercools to -12 degrees centigrade, plus or minus 1 degree centigrade. However, if the experiment is performed in the summer, the tail will freeze at -5.2 degrees centigrade, plus or minus 0.5 degrees centigrade. The suggestion is that this indicates that rat ancestors, like present-day insects, were more cold resistant in the winter. Interesting if perhaps unfounded.

G.J. Jones (Geneva, Switzerland) is trying to predict thermal histories of tissue samples during freezing (Abstract # 141). He believes that during slow cooling tissue will cool twice as fast as ice/water but that during rapid cooling tissue will cool twice as slowly as ice/water.

Finally, something about ischemic injury. The kidney is supposed to be devoid of hypoxanthine/xanthine oxidase, an enzyme implicated in causing a

cascade of free radical activity after an ischemic episode. There is now evidence that some form of this enzyme is generated in ischemic kidneys, evidently in connection with calmodulin activity (J. Lunec et.al., during presentation of Abstract #122). Perhaps this explains renal post-ischemic protection by allopurinol, and inhibitor of xanthine oxidase.

This concludes our coverage of the 20th Annual Meeting of the Society for

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Cryobiology. The summary of the finding presented at this meeting as they relate to cryonics is as follows. First of all, it is certainly encouraging that more papers than ever were presented on the subject of organ preservation. It is also encouraging that much more thought seems to be going into organ cryopreservation these days, and consequently some real progress is starting to be made on the level of understanding what is going on and what the best approaches are. Nevertheless, most organ preservationists are still not cryopreservationists. The number of competent labs studying this problem can be counted on the fingers of one hand. No laboratory seems to be studying brain cryopreservation or whole-body perfusion or freezing. Consequently, it should be clear to cryonicists that we are at a stage in history where the modest resources of a cryonics laboratory can make and really have to make substantial contribution to that part of cryobiology which concerns our survival. We can definitely make a difference. The overall progress being made in cryobiology as a whole, which we have reported here, provides us with a good theoretical and phenomenological background to draw on, as well as a good potential source of constructive criticism.

Additional research is the best path to improved prospects for survival. Cryonicists should recognize this fact and respond accordingly.

SIMPLE CRYOGENIC TECHNIQUES--PART II*

by Michael Darwin (Federowicz) and Hugh Hixon

ALARM PROTECTION FOR CRYOGENIC DEWARS

A pressing need which we have learned about the hard way is the need to have reliable alarm systems on patient dewars which can summon help immediately in the event of a vacuum failure, fire or other emergency. A variety of systems and sensors are manufactured to meet this need using radically different principles to sense an alarm condition. Basically, these sensors may be divided into two broad types: those which sense liquid level and those which sense temperature.

Liquid level sensors may operate on a variety of principles. Some use a hollow tube which is kept filled with gas by a small heating element inside the tube, under the liquid nitrogen. A pressure monitor or aneroid switch is connected to the top end of the tube and is set to close or open a circuit if pressure is lost in the submerged tube, or drops below a certain preset level. We know of no commercially available system of this kind, and a system which operates on this principle currently in use by Trans Time has performed acceptably, but is given to down time, false alarms and lack of precision in evaluating liquid levels.

A second approach to level monitoring is the use of a sealed capillary tube with or without a bulb containing nitrogen or another appropriate gas. So long as the bulb or the tip of the capillary remains immersed in liquid nitrogen, the pressure within the system will be very low.

Obviously, as soon as the tip is withdrawn from contact with the liquid, the pressure will rise. This system may

*The photographs which accompany this article appear in the center section of the magazine.

be coupled to an aneroid or microswitch and used to to trigger an alarm. The point at which an alarm condition occurs is determined by the depth at which the sensor is placed in the dewar. Generally, this depth or the point at which an alarm is sounded should be not less than half of the container's normal working volume. This allows for arrival of personnel before refrigerant has been exhausted and provides for safety margin for transferring patients and remaining liquid to a backup dewar.

Liquid level may also be sensed by the use of a temperature monitor wherein a thermocouple is placed at a fixed depth inside the dewar so that if the liquid level drops below that point the probe warms to vapor temperature and triggers an alarm. This kind of system is expensive (\$1,500 to \$2,500) because it requires the use of a cryogenic thermocouple thermometer/temperature controller.

The second class of alarms, those that work solely on the basis of sensed temperature may do so by sensing the absolute temperature inside the dewar and alarming on any significant deviation from a preset value. Alternatively sensors may be placed in the necktube, vent line, outer jacket or at the top of the cork, set to alarm if temperature drops significantly below preset values. All of these alarm systems, with the exception of absolute measurement of liquid level have inherent blindspots which can cause them to fail to alarm in a timely fashion, or fail to alarm altogether. Liquid level sensors dependant upon capillaries or gas filled bulbs which we have used, have sensitivities to temperature of about plus or minus 10 degrees centigrade before they alarm. This is not a serious problem if they are being used strictly as low level alarms to monitor filling or safeguard against a slow vacuum failure. However, in the event of a catastrophic vacuum failure, rapid boiloff of liquid nitrogen will virtually eliminate normal vapor/liquid temperature gradients and prevent the sensor from triggering the alarm until the tank has boiled dry and warmed at least 10 to 15 degrees centigrade. Temperature monitoring systems which depend upon the measurement of the "absolute" temperature inside the dewar (say -196 degrees centigrade) may also be "fooled" in this fashion as a result of a massive vacuum failure and rapid boiloff of liquid nitrogen.

External alarms which monitor jacket temperature or necktube temperature suffer from the reverse of this problem. If a vacuum failure occurs slowly boiloff may be greatly elevated without reducing jacket or necktube temperature enough to trigger the alarm. These kinds of sensors are also sensitive to swings in ambient temperature which occur if dewars are not kept in a temperature controlled environment (which they generally are not). These blind spots suggest a tactic of combining two or more different kinds of alarm systems to achieve more complete protection. This is what we have done. We selected a capillary type liquid nitrogen level sensor available from Almac Cryogenics (Model TOC-S) which consists of a copper capillary and a microswitch. The capillary is mounted in the lid of the dewar (photo G) and is set to trigger when the liquid level drops below the half full mark. A second sensor consisting of a Penn Model A-19 temperature controller is mounted in the lid, directly in the vapor vent path at the top of the necktube. This latter sensor is to guard against the possibility of sudden vacuum failure which the TOC-S capillary sensor

might be unable to detect. We believe this combination of sensors provides good protection against failure of the dewar.

A problem closely related to selection of alarm sensors is the selection of the notification system which the sensor is hooked to. Obviously, after the sensor detects a problem some means must be found to communicate the alarm condition to personnel who can then act on it. A wide range of systems for automatic dialing of telephones are available, and several have been adapted for cryonics use in the past. Experiences with the unreliability of these systems prompted us to look at professional equipment used to monitor households and

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businesses against fire and intrusion. The system we selected was a Napco Security Model 5000R automatic telephone dialer (see photo H). The Napco system is a ruggedly constructed device which allows for two separate tracks or classes of emergency messages. We use track A for dewar emergencies and track B for fire or intrusion. In the event both classes of conditions (dewar failure and fire or intrusion) occur at the same time, track B assumes priority, seizes the phone line and calls the police and fire department. ALCOR also retains a 24-hour answering service with a technician linked to the service by beeper (also on a 24-hour basis) to respond to emergencies such as vacuum failures. The dialer is programmed to dial the 24-hour answering service and instructs them to page the emergency technician on-call and inform him/her that there is a problem with the dewar(s).

LIFE EXPECTANCY AND PERFORMANCE OF SUPERINSULATED DEWARS

A critical question of interest to cryonicists everywhere is the issue of life expectancy and performance of high vacuum superinsulated equipment. Perhaps one of the first things we should discuss is our own experience and the experience of Trans Time with the use of superinsulated dewars. A major question in the use of these containers is their expected lifespan. Early on, when cryonics groups first began using MVE dual patient dewars we were apprised that the usual working life of a dewar was about 10 years at most. While this may be true for dewars used in the field where constant transport and handling abuse is common, this does not appear to be the case for storage dewars used in cryonics operations. It is certainly true that vacuum life is sharply limited on all dewars and that all dewars eventually "fail" for this reason. However, it is relatively easy and inexpensive to re-evacuate such "failed" dewars and restore them to previous levels of performance. In Trans Time's experience the average yearly cost per dewar for re-evacuation with four dewars in service is about \$350 per dewar.

One dewar now in service at Trans Time is 15 years old, and presents an interesting indicator of what the life expectancy of well constructed stainless steel dewars may be. This dewar, fabricated in 1969, by MVE was moved repeatedly throughout its working life, on several occasions with patients and liquid nitrogen in place. Total transport mileage for this dewar approaches if not exceeds 7,000 miles. The dewar suffered a total vacuum failure while in operation due to gross mishandling and required extensive rework of the necktube. Perhaps most interestingly, the dewar was maintained in nearly a foot of standing water for nearly six years of its working life. Today, 15 years later following appropriate re-evacuation and repairs this dewar is performing competitively with dewars more recently manufactured. It is becoming apparent that the lifespan of stainless steel dewars maintained under good conditions (i.e., protection from the elements and corrosive conditions) may be very much longer than

the 10 years originally anticipated.

In the nearly 10 years of continuous operation experienced by the dewars currently owned by Trans Time, only two actual "leaks" which required repair have developed. Both of these were repaired with relative ease and modest expense, and one was related to transport of the dewar.

Where the question of efficiency is concerned, it is worth pointing out that superinsulation is hard to beat. This is particularly true if space is at a premium--and space costs money! Current MVE dewars perform at a boiloff rate of about 6 liters per patient per day. Current costs for such a dewar from the manufacturer (without interior support structures, casters and so on) is about \$8,000; or \$4,000 per patient. With a small pool of whole-body patients the use of dual patient dewars probably represents the best approach because of the

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flexibility and ease of handling it offers. If the number of stable, well funded whole-body patients grows, it will rapidly become more economical to use dewars with better surface to volume ratios and go to a multipatient approach to storage. ALCOR has investigated the costs of constructing such a system (with a reliable manufacturer) and has found, perhaps not surprisingly, that the cost of superinsulation per patient is relatively constant and that a dewar capable of holding up to 10 patients (depending upon their size) would cost approximately \$40,000 or about \$4,000 per patient--the same cost per patient as experienced with an MVE.

However, liquid nitrogen usage in such a system would be only 35% per patient of that experienced with a conventional two patient MVE. Short of undertaking the project ourselves, it would be impossible to know how the capital expenditure for a superinsulated dewar would compare to the development of an in-house prototype with the same capabilities. We suspect that the cost of an in-house system would greatly exceed the cost of a system manufactured for us. Hopefully time will tell if this is the case, and we look forward to evaluating the experiences of others in grappling with these problems.

MISCELLANEOUS OBSERVATIONS

Use of Wood

With resources being so scarce in cryonics, cost effective fabrication of structures for use in dewars is no small problem. Custom sheet metal work and heavy slabs of aluminum and stainless steel are very expensive. In the past patient trays and support structures have been made from these kinds of materials. If there is one thing we have learned in recent years, it is that this approach is the WRONG approach. Common wood works beautifully at liquid nitrogen temperature and appears to suffer no loss of strength. A length of wood cooled to liquid nitrogen temperature will ring like a bar of steel if it is dropped on the floor. It is greatly hardened by cooling to such extremes and becomes about as resistant to penetration by a nail as a comparable thickness of aluminum. Well over a year ago ALCOR switched to wood for use in patient support structures such as stretchers and internal rockers. The realization that wood was safe to use has opened up a whole range of engineering options which were previously closed to us because of prohibitive cost or the need for expensive machine shop equipment. The use of wood has also greatly reduced the expense of the dewars we're using and improved their flexibility in ways we are only now beginning fully to appreciate.

In the past, MVE dual patient dewars came equipped with a siderail

assembly designed to accommodate two patient stretchers. This assembly required that numerous cross supports be welded into the inner cylinder and that special teflon-lined tracks be fabricated to allow stretchers to be moved in and out. This system has a number of drawbacks. First, it is costly: it is estimated that had we used this system it would have raised the cost of our dewar by nearly \$2,500 or over 30%! Instead, we fabricated two trays out of 3/8" plywood using aluminum angle on the edge for stiffening. A special rocker assembly was constructed of 1/2" plywood in the bottom of the dewar (see photo I), which was designed to guide the stretchers into a common groove when they were lowered into the dewar via a crane. Once in position, the first stretcher can rock forward a distance of as much as 5" or 6" (depending on the dimensions of the patient on it) to allow accommodation of a second obese or abdominally distended patient through the necktube which is three inches smaller in diameter than the inside of the dewar (see photo J). In sharp contrast to the fixed

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stretcher/rail system previously in use, this system allows for tremendous flexibility in loading patients into the dewar. Once patients are in place, wooden braces are used to immobilize the stretchers.

Wood also has the advantage of being (even in its cold, hardened state) unable to puncture the inner cylinder during loading, handling or moving operations. In the future, when intermediate sized (10 to 20 patient) multiple storage units are put into operation, wood may well be used to fabricate protective boxes or cassettes for whole-body patients.

Packing Materials

We have tested a variety of other packing and handling materials and found a fair number of things to hold up well. Cotton, polyester, nylon and most synthetics do very well at liquid nitrogen temperatures. Dacron or so-called polyester wool is an outstanding material for packing and insulation as it loses neither its loft nor its flex properties with prolonged liquid nitrogen immersion. When using Nylon sear fabrics (the type windbreakers and parachutes are made out of) it is important to make sure the fabric has not been made waterproof by impregnation with urethane or Gortex as it will stiffen and crack at liquid nitrogen temperatures. As these techniques are increasingly used on products, we are finding it harder and harder to find acceptable Nylon gear and have moved increasingly to polyester and cotton polyester products. For protection and retrieval bags used on neuropreservation patients we have begun using cotton/polyester "flannel" pillow cases in place of previously used Nylon sleeping bag "stuff bags" (see photos K, L and M).

A good insulating material with outstanding absorbent properties is common open cell urethane foam. Closed cell foams such as Ensofoam should be avoided because of their unfortunate tendency to become brittle and absorb liquid nitrogen over long periods of time. The latter property results in explosive "popping" of the material when it is removed from nitrogen. Dacron wool, as previously mentioned, is also a good insulator, but it does not hold liquid nitrogen well and thus does not provide the heat sink protection during transfer operations which may be had with open celled urethane foams.

SUMMARY

We realize we have treated a large array of problems here. We hope that this kind of article will stimulate others to expose the problems they have encountered and any solutions they have developed. Certainly our list

of technical difficulties is not complete and hopefully we will have the opportunity to share more information like this in the future. We wish to encourage others to do so as well.

Special thanks to Frank Rothacker and to Art Quaife of Trans Time for the information they provided and for the solutions to more than a few of the problems we have discussed here.

LIFE EXTENSION FOUNDATION MAKES GENEROUS RESEARCH CONTRIBUTIONS

We are pleased to announce that ALCOR has received a \$5,000 research contribution from the Life Extension Foundation in Hollywood, Florida. The nature of the research the contribution is to be used for has not yet been established. In the next month or so we plan to submit a proposal to LEF detailing our plans for the contribution. In the meantime, the money will remain in the Research Account untouched until an agreement about how to spend

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it is reached with LEF.

We understand that LEF also made similar contributions to the Institute for Cryonics Education (ICE) as well as to the Bay Area Cryonics Society in support of Paul Segall's hamster work.

We would like to thank the Life Extension Foundation and Saul Kent and Bill Faloon for their generosity.

NEW ALCOR WALLET EMERGENCY CARDS

URGENT INSTRUCTIONS

THE BEARER OF THIS CARD IS AN ANATOMICAL DONOR UNDER SECTION 7153.5 (A) OF THE HEALTH AND SAFETY CODE OF CALIFORNIA AND HAS MADE ARRANGEMENTS FOR POST MORTEM CRYOGENIC PRESERVATION UNDER SECTION 7100 OF THE HEALTH AND SAFETY CODE OF CALIFORNIA. PLEASE FOLLOW THE INSTRUCTIONS BELOW.

1) IF UNCONSCIOUS, SERIOUSLY INJURED, OR CLINICALLY DEAD, IMMEDIATELY CALL (COLLECT) 714-738-5569 AND NOTIFY ALCOR FOUNDATION. STAY BY THE TELEPHONE TO RECEIVE INSTRUCTIONS. ALCOR PERSONNEL WILL BE PAGED.

2) IF CLINICALLY DEAD, START AND MAINTAIN CARDIOPULMONARY (HEART-LUNG) RESUSCITATION (CPR).

3) MAINTAIN ARTIFICIAL CIRCULATION AND VENTILATION AND ADMINISTER APPROPRIATE PARENTERALS TO MINIMIZE ACIDOSIS.

4) IF LEGAL DEATH HAS BEEN PRONOUNCED AND IT IS POSSIBLE TO DO SO. CONTINUE CPR AND PACK THE BODY IN ICE (ESPECIALLY HEAD, THROAT, AXILLA AND GROIN) OR PLACE ON A COOLING BLANKET AT 2 TO 4 DEGREES CENTIGRADE.

5) IF EXTENDED CPR IS NOT POSSIBLE OR INAPPROPRIATE (DONOR DEAD MORE THAN ONE HOUR) PACK THE BODY IN ICE.

6) DO NOT UNDER ANY CIRCUMSTANCES ALLOW THE BODY TO FREEZE OR BE EXPOSED TO SUBFREEZING TEMPERATURES (i.e., BELOW 0 DEGREES CENTIGRADE OR 32 DE-

GREES FAHRENHEIT).

DO NOT AUTOPSY OR EMBALM.

Call ALCOR (714) 738-5569 DONOR NUMBER _____

The new ALCOR EMERGENCY WALLET CARDS pictured above are now available. We will be mailing one of these to each of our General Members. Please fill in your Donor Number in the blank provided for this purpose on the front of the card. Additional cards are available to ALCOR General Members for twenty-five cents apiece.

We hasten to point out that you should NOT use these wallet cards instead of your bracelet or necktag. Wallet cards are very unlikely to be found in the event of an emergency and are provided only as a source of additional information. You should put your wallet card in the same pocket as your driver's license or otherwise place it near similar key piece(s) of identification in your wallet or purse.

We have included the statement of anatomical donation for several reasons: 1) the Attorney General's opinion is just that, an opinion, 2) the "opinion" says whole-body cryonic suspension does not qualify under the Uniform Anatomical Gift Act(UAGA) because it cryonic suspension is not for the express purpose of transplantation of tissues, 3) point 2 may well exempt neuropatients (who ARE tissues for transplantation).