CRYONICS

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Contents:

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EDITORIAL MATTERS

In response to our recent public service campaign about the importance of wearing cryonics I.D. tags we have received several requests for bracelets/necktags from BACS members. We are gratified by this response, but unfortunately, ALCOR tags are considerably different than BACS tags and are available only to ALCOR General (suspension) Members. If you are a BACS member and need a new neck tag or bracelet you should contact BACS and they will order one for you through Medic-Alert.

As discussed elsewhere in this issues (see articles on pages 4 and 13) recent research has yielded an improved heat exchange medium for cooling patients to dry ice temperature. The many disadvantages of isopropanol are summarized in both of the other articles. Suffice it to say that there is a clear advantage to the use of Dow Corning 200 (5 cs) silicone fluid over isopropanol. However, this presents us with us a problem (see "Equal Unfairness" on page 5): ALCOR can simply not afford the $3,000 expenditure

required to purchase enough of this material for whole-body patients. Owing to the generosity (and self interest) of Hugh Hixon and several other neuro-members, ALCOR now has 5 gallons of the fluid on hand: enough for neuropatients. This does not, however, solve the problem for the seven whole-body members.

All we can do under the circumstances is to go to you, our whole-body members and to our membership as a whole and ask for financial support for purchase of the material. It is probably wise to point out here that even neuro-members may benefit from whole-body members acquiring enough DC-200 to switch from alcohol as a cooling medium. Why? Because at this time we have 65 gallons of isopropyl alcohol sitting in Cryovita. In the event of an earthquake the metal drum containing the isopropanol could quite possibly rupture, allowing large amounts of of flammable liquid to spill out into the facility. This presents no small fire hazard. Indeed, 65 gallons of flammable liquid in or out of a metal drum is not exactly something either cryonicists or insurance people like to have sitting around a patient storage facility. So, the sooner we get rid of the isopropanol, the better.

The whole issue of the silicone cooling fluid raises the question of "equal treatment." Is it possible for ALCOR to offer the same degree of protection and the same quality of services to its whole-body patients as it does to its neuro patients?

The answer to that question is:

No.

Nor should this provoke shouts of "unfair" or "inequitable." It is a simple fact of logistics, engineering, and economics that whole-body patients cannot be given the kind of mobility and protection we soon hope to afford neuropatients with our cephalarium vault. Additionally, physical law constrains us as to the cooling rate achievable with whole-body vs. neuropatients. There is nothing we can do to change these situations short of greatly increasing (the already unequal) fee for whole-body suspension.

Perhaps most important, it should be pointed out that neuropatients have given up some advantages to get some. It is all well and good for whole-body people to shout: "unequal treatment" when they have not had their bodies cremated. The fact of the matter is that neuropatients are sacrificing whatever advantages may accrue from having their bodies and their intact spinal cords make the trip with them. They have given up precisely what whole-body people are unwilling to give up in order to secure some special privileges: mobility, reduced cost of perfusion, cooling, storage, legal freedom, and ease of daily care and handling. By their own choice whole-body patients have traded these advantages away in favor of carrying their entire bodies into the future with them.

With this perspective in mind, perhaps charges of "unfair" or "unequal" don't seem so serious. Suffice it to say that ALCOR cannot treat whole-body and neuropatients with precise equality. Also, suffice it to say, that ALCOR will endeavor to DO THE VERY BEST IT CAN for ALL its members and patients: whole-body or neuro. Anyone who expects or demands more should be prepared to pay for it.

NEW PERFUSATE FORMULATION

Since the early 1970's the base perfusate used in cryonic suspensions has been undergoing a steady evolution. Initially, this evolution was directed primarily by armchair research in the form of looking at the cryobiological literature and extrapolating to our circumstances. After

about 1973, cryonicists began conducting their own research to evaluate the best perfusate formula, and since 1977 we have had definite criteria for making changes, namely that changes must be shown to be a substantial improvement in terms of cost and/or ease of handling and must be at least as good or better than the preceding formulation at supporting viability. We have also required in-house tests of the perfusate's compatibility with the cryoprotective agent currently in use. Further in-house testing is required to insure that the perfusate can be used safely in an intact animal, i.e, during perfusion. This latter requirement is to insure that a perfusate which checks out well with tissue slices be shown not to cause serious edema or other gross side-effects which might not become apparent until it is actually perfused.

All of the perfusates in use since 1974 have been "glycerophosphate-based" or in other words, have gotten a large fraction of their buffering (acid neutralizing) and/or osmotic (water holding) activity from sodium glycerophosphate, a sugar-salt. The use of glycerophosphate-based perfusates was a tremendous advance over previously used "simple" electrolyte solutions such as Ringer's solution. Indeed, the use of glycerophosphate has in part been responsible for allowing closed circuit, cryoprotective perfusion to proceed for as long as 7 hours in ischemic human patients before edema (tissue swelling) becomes a limiting factor.

There are, however, some problems associated with the use of glycerophosphate- and phosphate-based perfusates. Recent research conducted by Buckberg, et al (1) has demonstrated the need for "high" pH (8.0 or higher) in the range of 10 to 15 degrees centigrade. Unfortunately, phosphate buffers such as those employed in a glycerophosphate-type perfusate cannot easily remain in solution at this high of a pH. The result is precipitation of these chemicals which in turn causes unstable perfusate composition and loading of filters during perfusate preparation. Also, the phosphates tend to take magnesium and calcium with them when they precipitate out of solution. Stable magnesium, calcium and phosphate levels are essential to good viability and adequate perfusion.

For these reasons, about a year ago ALCOR began experimenting with synthetic organic buffers: principally HEPES (which is short for N-2-hydroxyethyl piperazine-N'-2-ethane sulfonic acid). HEPES, unlike glycerophosphate, is compatible with high pH's and does not cause precipitation of calcium and magnesium salts. HEPES is also compatible with good tissue viability and a formulation very similar to the one we've chosen to replace our glycerophosphate based perfusate is now in use at the Red Cross Blood Research Lab by investigators working on kidney preservation, with similar good results.

Our own tests in perfusing cats and rabbits with this perfusate indicate no untoward gross effects. Perfusate mixing has been greatly simplified by substitution of the HEPES and filter loading has been substantially reduced as well.

The formula we are currently using is given below:

MANNITOL-HEPES PERFUSATE FORMULATION

Dextran 40		50g/l
Mannitol	170.0mM	30.97g/l
Glucose	10.0mM	1.80g/l

HEPES	7.2mM	1.72g/l
Glutathione	5.0mM	1.54g/l
Sodium Bicarbonate	10.0mM	0.84g/l
Adenine HCl	l.0mM	0.17g/l
Potassium Chloride	28.3mM	2.11g/l
Calcium Chloride	l.0mM	0.5ml of 22.2% solution
Magnesium Chloride	2.0mM	1.0ml of 40.66% solution
Sodium Heparin		1,000 units/l

We print this formula as a point of information for our own members. We wish to emphasize that this does not constitute a recommendation to other cryonics organizations. Each organization should carefully evaluate perfusate formulations in-house before changing their perfusion protocol.

WHAT YOU CAN DO, IV: ONE BACS GOVERNOR'S VIEW by John Krug

The following remarks are some of my thoughts on what Northern California members of the Bay Area Cryonics Society and others can do to help the growth of cryonics where we are. These suggestions should not be construed as the official policy of BACS and are only the views of myself, one longtime and concerned cryonics advocate.

The first thing a BACS member could do after making complete legal and financial preparations for cryonic suspension is to give continuing associate memberships in BACS to their next of kin not living at the member's home. At

the same time, giving gift subscriptions of cryonics periodicals to relatives and friends would be a good way to let our intentions be known and serve an educational purpose as well.

Another project that could be considered is to bring cryonics to other special interest groups the member is involved in. As recent regular readers may know, I have been active in the L-5 Society, a growing organization of 10,000 members which promotes the development of human settlements in space. I was disappointed that I was unable to interest my colleagues in BACS or Trans Time in presenting cryonics to the Third International L-5 Conference which was held in San Francisco over Easter weekend. In spite of this lack of organizational support and my own awkwardness, I was able to make some notable contacts there -- including a U.S. Congressman who was the keynote speaker at the conference banquet.

While I was trying to get support for cryonics participation at the L-5 event, I found that a couple of the other members who are on the BACS Board prefer to do radio and TV spots as publicity. I think think this approach, if done to the exclusion of others, is depersonalizing and elitist. As far as I'm concerned, I'm not too good to engage in intelligent dialogue with potential allies in the struggle against deanimation. I sincerely hope that cryonics groups will do presentations and panels at future events sponsored by L-5 and other similar open-minded organizations.

An additional concern of mine is how we handle the much sought after

mass media publicity. In my view such media efforts should be done only after prior consultation and approval of the the sponsors and next of kin of featured suspension patients. I am against cryonics publicity that exhibits suspension patients in less than a dignified manner. This almost happened a couple of years ago when an escape artist wanted to do a publicity stunt in a "That's Incredible" vein at the Trans Time facility. Although the project was cancelled for logistic reasons, I remain concerned that similar programs will be undertaken in the future. I think that if cryonic suspension is to grow as an option for most people, it would be wise to keep our promotional efforts on a professional and educational basis. The patients as well as the general public deserve a generous degree of respect.

Another matter I've been thinking about is the neuropreservation or "heads only" option of suspension. As some of you may know, I am against it at least for myself on grounds that it may be less than scientific. I don't think that this mode of suspension can be dressed up in a pretty package and sold to the public. I think that efforts, both technological and promotional, should be made to make the whole-body method affordable. In the meantime, neuro advocates could help matters by developing and funding within existing cryonics groups to pursue human cloning research programs necessary for this option to be a success. I don't think that anyone outside our circles will do this or be obliged to pay for it.

I think the editors of CRYONICS deserve the thanks of all of us for about four years of consistent publication. Let's hope their efforts will continue.

John B. Krug
BACS Governor

EQUAL UNFAIRNESS

The ALCOR Board of Directors met on 6 May, 1984. As usual, most of the items on the agenda were disposed of expeditiously. With one exception, which

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is still annoying me.

After a considerable amount of effort, Mike Federowicz, President of ALCOR, has managed to find a heat exchange liquid which if not perfect is at least a major improvement. To recap on this remark a bit, after the perfusion portion of a suspension is complete, the patient must be cooled for long-term storage. This is usually done in two steps: first, from about 10 C to -79 C (dry ice), and; second, from -79 C to -195 C (LN2). Since a considerable amount of unwanted biochemistry can still take place above -79 C, it is considered best to do the first step reasonably quickly. Dry ice comes in blocks. Patients come as, well..... patients. A heat transfer medium is required to cool the patient with the dry ice. This can be either a gas or a liquid. Gases are not particularly effective due to their low density. This leaves liquids. Up to this time, the cooling liquid for the first step has been isopropyl alcohol (isopropanol). Isopropanol has several advantages which led to its selection in the early days of cryonics. At room temperature it is a liquid. At dry ice temperature it is still a liquid. It is cheap. Isopropanol also has some disadvantages. It should not be allowed to contact the patient directly, since it can leach out the water and ice in the patient's tissues. In practice, it is damn near impossible to keep the isopropanol out of the protective bag, but we do try. Isopropanol is

rather flammable, and presents a storage hazard. It is moderately poisonous.

With these and other disadvantages in mind, Mike as noted above has spent several years looking for a substitute. His search is his own story, but to summarize his work, the silicone fluid Dow Corning 200 (5 centistokes) is liquid over the required temperature range, can be cleaned up and reused, does not leach out water, is non-toxic (except perhaps by drowning), and is relatively non-flammable. At $6.07/lb, it is, however, a bit expensive. A neuropreservation would require about $300 worth, and a whole-body suspension about $3,000. This would be essentially a one-time expenditure, but the money has to come from somewhere. Hence the item on the ALCOR agenda.

As presented, the agenda item was roughly thus: To buy or not to buy five gallons of DC 200(5cs), to be used for large-scale final testing and for any future neuropreservation suspensions. The purchase of the larger amount that would be needed for a whole-body suspension was considered only in passing for two reasons: Mike didn't need that much for his tests, and besides, ALCOR couldn't afford it.

At this point, Jerry Leaf, board member and whole-body candidate, raised an ethical objection. Since ALCOR is committed to the equal treatment of each and every one of its suspension members, the purchase of only enough DC 200(5cs) is unfair to the interests of the of ALCOR's seven whole- body candidates. This being the case, enough liquid should be purchased for a whole body suspension, or none at all. He got quite adamant about this.

Time out to examine the "equal treatment" idea.

There is a very sound reason for this policy. If the members of a group are treated unequally, and particularly if there is no rationale to an unequal treatment, the usual result is that the group splinters. Given the number of cryonicists in the world, this is usually disastrous for those concerned. The splinter groups fall below the minimum number to accomplish a suspension, and no-one gets anything. To paraphrase Benjamin Franklin, by not hanging together, we hang separately.

There are also good reasons why equal treatment may be impossible to implement. For rapid response to a deanimation, ALCOR has an effective operating radius of about 100 miles away from the Los Angeles area. Money and the time available to the members responding to an emergency voluntarily are the major constraints. ALCOR also has suspension members in places like San Luis

Obispo, Las Vegas, Lake Tahoe, Maryland, and Australia. I submit that given ALCOR's less than infinite resources, these members cannot be treated equally with those in the LA area, much as we would like to do so. However, I also submit that the people in question, not being fools, were aware of their natural disadvantage in this respect, and decided to take their chances with ALCOR anyway, equal or not.

Having determined that, from time to time, ALCOR may have no choice but to be unequal, we now return to the question before the Board.

I recast the question as follows: Should ALCOR purchase for final testing an amount of DC 200(5cs) which will also be enough for a neuropreservation suspension. This will improve the treatment of the neuropreservation candidates, but will not benefit the whole-body candidates. Or, should purchase be deferred until ALCOR can afford enough DC 200(5cs) for a whole-body suspension, a mere matter of $3,000. Until such time, no-one to benefit.

Some alternatives were suggested, such as assessing each group (neuropreservation and whole-body) a proportionate share of the cost. My

own suggestion to the Board was that it approve the small purchase, with the understanding that we were being unfair to the whole-body candidates, but would correct this when we were financially able to do so. By this time, however, the meeting had degenerated to a sort of Greek chorus, Jerry Leaf on one side repeating "equality," and the rest of the Board answering antiphonally, "buy some," but unwilling to step on Jerry and get on with it.

While I am definitely in favor of fairness, and dislike its absence, I have an even greater dislike of indecisiveness in general, and stupid indecisiveness in particular. At about the 20 minute mark in the debate, I got annoyed enough to do something personally expensive, and said, "I'll buy it." End of debate.

This has now been done.

I criticize the Board as follows:

Jerry Leaf for getting so locked into a philosophical principle as to suggest that we cut off our noses to spite our faces, so to speak.

The rest of the Board, for not politely stepping on Jerry and making a decision which would have in fact benefitted a clear majority of the suspension members, and for not making some commitment to the remainder of them.

I would like to thank the following people for spontaneously contributing toward the purchase of the DC 200(5cs). Mike Darwin, Maureen and Paul Genteman, Anna Tyeb, Bill Jameson, and Carlos Mondragon and Arthur McCombs. As I am some distance from breaking even, I will not discourage other contributions.

Hugh Hixon
Member, ALCOR Board of Directors

In liquid nitrogen
the minds rest.
Dreamless.
Without life.

We carry them to the future
like children.
On our shoulders.
And in our hearts.

--Hugh Hixon

LAKE TAHOE LIFE EXTENSION FESTIVAL: A GREAT WEEKEND! by Mike Darwin

While the Life Extension Festival did not officially "open" until Saturday, May 26th, at 4:30 PM, some of us lucky souls got away a day early and arrived in Lake Tahoe on Friday evening. In order to accommodate the earlybirds, Fred and Linda Chamberlain hosted an evening spaghetti dinner and get-together at their home. The Chamberlains, organizers of the Festival and founders of ALCOR, live nestled away in the wilderness in a sort of picture book cottage. One expects to see Hansel and Gretel come

strolling down the lane almost any time. Indeed, one could easily become as lost as Hansel and Gretel trying to find the Chamberlain's home.

I was lucky: I rode up to Tahoe with Fred's son, Fred IV, and so we only a took a couple minor wrong turns on our way through the forest! Keep in mind the younger Fred was going home and even he took a wrong turn or two! After our long drive and longer trek through the forest we arrived to a lovely meal and the good company of fellow immortalists. With the weekend off to a good start we adjourned to our respective motels around 10:30 PM and prepared for the May meeting of the Bay Area Cryonics Society which was to be held the following morning at 11:00 AM.

However, a few of us adventurous souls had earlier plans for the morning. The Chamberlains managed to lure three other hardy people out of bed for an early morning run along Fallen Leaf Lake. I was definitely prepared for the worst as I went to bed on Friday night. I am a night runner, which means an early run for me is 5:00 pm! The thought of struggling out of bed at seven in the morning in a place where the air is so thin you can't even see it (Lake Tahoe is a little under a mile high!) to a likely 45øoF was almost more than I could bear to think about! The thought of having to compete against two seasoned veterans like Fred and Linda who are used to conditions a lot like a good day on Mars at the equator at noontime really had me worried!

Much to my surprise I woke early on Saturday feeling refreshed and ready to go. The temperature was a balmy 50øF or 55øF and the sun was up in a crystal blue sky framed by the gorgeous snow-capped mountains which rim the lakes. Promptly at eight the Chamberlains arrived to pick up Saul Kent, Al Lopp and myself for the trip out to Fallen Leaf Lake. Lest I go on for pages and pages, suffice it to say that the run was wonderful. I did a fairly easy five miles or so over moderately hilly terrain. My only complaint was my hands and ears: they simply froze in the chilly, tree-shaded morning air. Next time I'll bring gloves and a cap! We wound up the run next to what the Chamberlains described as "kind-of-a-waterfall." "Kind-of-a-waterfall" turned out to be a several hundred feet drop of rushing white water which was anything but "kind of" a water fall. God help us if we ever encountered what the Chamberlains must think of as a real waterfall! All in all it was a delicious morning: crisp clean air, sunny, bright blue skies, a good workout with good friends and "kind-of-a-waterfall."

Following the run we attended the BACS meeting which was held at the Tahoe Marina Inn, a couple of doors down from the Timbercove Lodge where the Festival was to be held. The BACS meeting started at 11:00 in the morning and ran through to nearly 4:00 in the evening! For those who have attended BACS

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meetings previously, this one should have come as no surprize. It is difficult for me to frame comments on this meeting. Being President of ALCOR anything I say which is not frankly positive will be interpreted as unfair criticism and harsh negativism. ALCOR has been accused of being too autocratic and not democratic enough: too quick to arrive at decisions and not careful enough evaluate the opinions and desires of all of its members. No doubt there is truth to these comments: ALCOR believes in management decisions for most day-to-day administrative affairs. There are strengths and weaknesses to this position.

On the other hand, the BACS meeting left me wondering how anything gets done in Northern California. Discussion on small points was vituperative

and seemingly interminable. Officers and Board members were frequently flatly uncooperative or frankly insubordinate, in one case refusing to turn information requests over to the president on a timely basis so that they could be promptly answered. In such an atmosphere of hostility and noncooperation it is difficult to envisage how big decisions which require coordination and good communication can be made. A number of serious issues on the agenda such as where BACS patients will be stored when Trans Time's lease runs out this Fall were simply left unresolved, while smaller issues, such as who should pay for the new BACS flyer (which John Krug had printed up) were debated almost endlessly until resolved.

Of particular concern to ALCOR is the issue of patient records. ALCOR has repeatedly asked BACS and Trans Time for complete medical and patient care records on two ALCOR suspension patients which BACS formerly cared for. Despite a recommendation by BACS attorney James Bianchi in his BACS legal checkup that such records be compiled and maintained for each patient, BACS Secretary/Treasurer Paul Segall stated that such record keeping may well be beyond BACS's administrative ability. Despite the fact that the issue of complete records for patients has been raised repeatedly with BACS and was again brought up by me at this meeting, we have been given NO indication of when, if ever, BACS plans to provide ALCOR with these records. In one instance, BACS/Trans Time has lost the cooling data on one patient ALCOR now has responsibility for.

One thing was painfully clear from this meeting: BACS has a large number of tough decisions facing them. Decisions which cut to the core of how they will pursue services for both their living members and the patients they currently have in their care. Everywhere during the course of the meeting decisions were being weighed against the impact they will have on BACS's principle service provider, Trans Time. Very serious issues of potentially great liability such as patient record keeping, standards for handling prepayment of suspension monies, and the future security and care of suspension patients loom on the horizon.

ALCOR is certainly not free from many of these problems or concerns. We understand the difficult tasks which confront our Northern neighbors. We urge BACS to achieve a prompt resolution to the more immediate problems facing them, and to be mindful of their obligation to the patients--both those they are now responsible for and those for whom they have been responsible in the past. Sorting out priorities is often a tough thing, particularly in the thick of battle. We urge BACS to put the patients first, and among other things to begin doing everything necessary to insure their well-being, including assembly and maintenance of good medical records.

On a more positive note, it was revealed by BACS researcher/treasurer Paul Segall at the meeting that he had received assurance for funding from a "Texas based individual of considerable means." This individual, and an organization he represents have reportedly pledged $5,000 per month in support of Paul's

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hamster perfusion project: such support to be forthcoming for at least a year. Our understanding from the meeting was that this support was to be channeled through BACS to a for-profit company owned by Paul and Dr. Harold Waitz known as BioPhysical Research and Development (BPRD). Presumably BPRD and BACS will have a contractual relationship assigning patents and/or profits on an equitable basis between the two concerns.

Probably the most valuable thing to arise from this meeting was BACS attorney James Bianchi's "legal checkup" report. BACS' peculiar problems entirely aside, this report was a model checklist of legal considerations for anyone in any way responsible for running a small organization. Mr.

Bianchi is to be commended for a valuable and comprehensive survey, and BACS for commissioning same.

Following the BACS meeting everyone rushed off to get ready for the horseback ride and barbecue which was to start at 4:30 PM at Fallen Leaf Lake. I had not been on a horse in over ten years. I have NEVER been on a horse on mountain trails. In short, I had no idea what to expect. I thought I was going to get a little 45 minute pony ride through a nice little wooded trail. About an hour into the forest I asked the guide how much longer we had to go. "Oh, I guess about two hours more," she replied! While it was a fairly easy ride, it was a lot more interesting one than I had imagined. We ascended the mountains ringing Fallen Leaf Lake to about 8,000 feet, moving along a steep, rocky trail. One serious misstep there and you were in for a long, hard tumble! My sense of ease was not helped by noticing that the horses were stumbling on the rough trail from time to time or by my own beast's inclination to stop for lunch with frequency--even if it meant a little jaunt through low hanging branches into the underbrush! Nevertheless, I was lucky: Hugh Hixon got a horse which was quite mean and occasionally snapped and charged at its neighbors. So, despite my horse's inclination to snack, it at least didn't act like it had rabies.

Perhaps because the length and "ruggedness" of the ride so surprised me, I think it was the high point of my "recreation" during the weekend. The scenery was simply breathtaking, with massive snow-capped mountains peeking through towering conifers. Our trail took us along the shore of the crystal-blue waters of Fallen Leaf Lake up 3,000 feet into the heavily wooded, rocky mountainside. All of Lake Tahoe is enchanting: it is just storybook country. The Chamberlains call their business Paradise Rentals and they answer the phone "Paradise." One trip up there and you'll know why. At every turn on the horseback ride I expected to see Little Red Riding Hood dart out of the woods. Certainly, deep in the forest wilderness the Big Bad Wolf would have been right at home. Lucky for "Slick" (my horse) and I we didn't see him, or we probably would have ended up a snack instead of just stopping for one.

Sunday morning at 10:30 AM the "Technical Sessions" began with a talk on the laws of cloning by James Bianchi, Northern California "cryonics attorney." As usual, Jim's talk was lively and easy to comprehend. Basically, Jim pointed out that there are a number of laws extant which cover the rights of fetuses to life and health. As was made clear from Jim's presentation, cloning for transplantation, or other purposes which might be construed as "detrimental" to the life of the fetus are severely restricted by law. Any attempts to clone for such purposes must insure that the fetus meets criteria for brain death at the time of birth, and further that any "detrimental" manipulation of the fetus (such as destruction of the brain) must be carried out very early in fetal development, before a spontaneous heartbeat occurs. My brief account here can in no way sum up all the fine points Jim made in his presentation. Hopefully, he will be willing to write up an article detailing his legal researches for a future issue of CRYONICS.

The next paper was presented by me and was entitled "Silicone Liquids as

Heat Exchange Media." I'll not discuss this paper at all here, since it is printed in full elsewhere in this issue. This will also be the case with several other papers which were presented on Sunday as they will soon be presented in full in the pages of CRYONICS over the next few months. As most of these papers are already written and "ready to go" we hope to be able to keep our promise this year of being able to bring you full reports

of many of the papers presented.

Jerry Leaf followed with the next two papers: one on hollow plastic spheres as a floating insulation blanket for inside liquid nitrogen dewars and one on the "Real Price of Water for Perfusion." The latter paper was a thoughtful analysis of the costs associated with various methods of producing U.S.P. quality water, with special attention to the risks and problems associated with "do-it-yourself" schemes.

While conclusions from Jerry's first paper were still in doubt, the upshot seemed to be that floating plastic spheres do not significantly reduce boiloff. Jerry plans to repeat the work with a larger thickness of the spheres to see if there is any improvment.

Jerry's third paper documented the perfusion and initial cooldown of two patients placed into suspension in 1980. One of these patients was recently converted to neurosuspension and the remains autopsied. Jerry's report on the preparation of this patient was thus of special interest since a discussion of postmortem findings was to follow in the afternoon session. Jerry's paper should be ready for publication within the next month or two.

I started off the afternoon session with a report on the postmortem examination of the remains of three whole-body suspension patients who had been converted to neurosuspension. A number of interesting and quite unexpected findings were made as a result of these autopsies. Information gained from these examinations may have a profound impact on cryonics procedures in the future. A full report of these findings will be presented in the August issue of CRYONICS.

Following me, Greg Fahy presented a report on the histology of one of the suspension patients who had been autopsied. Greg's report was both comprehensive and very exciting and we hope to present a written version in August or September.

Greg then followed with a paper on the "Cellular Structure of Frozen vs. Vitrified Brains." I think everyone present for Greg's presentation was impressed with the quality and thoroughness of his work. His slides of rabbit brain gross structure following straight freezing, cryoprotection and freezing, and vitrification were simply breathtaking. One thing is certainly clear from Greg's work: gross structural preservation of the central nervous system following glycerolization and freezing is outstanding. Material from both the cerebral cortex and cerebellum showed clear, crisp cell membranes, intact myelin sheath, excellent preservation of cell-to-cell relationships and intact axons unmolested or severed by ice crystals. Occasionally, some gaps or holes were seen in the neuropil (the weave of axons and dendrites) which looked like artifacts of ice formation, and occasionally cell shrinkage (apparently as a result of dehydration secondary to cryoprotectant introduction) separating neurons from connecting dendrites could be seen. But these kinds of changes were the exception rather than the rule. Overall, brains glycerolized to 3 Molar, cooled to and rewarmed from -79oC were almost histologically normal. As Dr. Fahy himself summed up: "histological preservation in frozen-thawed brains was breathtakingly normal!"

Unfortunately, brains treated with vitrifiable amounts of cryoprotectant agents did not fare so well. In many instances the cell nuclei appeared separated from the cytoplasm and the cytoplasm had a somewhat grainy appearance.

There was also much more evidence of cell shrinkage, perhaps as a result of the high concentrations of cryoprotective agents (over 50%) required to achieve vitrification at normal atmospheric pressure.

Greg's final paper entitled "New Advances in Vitrification" briefly

reviewed the nature of vitrification. Greg pointed out that vitrification, as opposed to freezing, does not involve ice crystal formation or a "phase change." In other words, what occurs when vitrification takes place is that the solution gets thicker and thicker until it solidifies into a glass. With freezing there is an abrupt transition from liquid to solid with highly organized ice crystals occurring. Because vitrification avoids crystallization it allows escape from all the harmful side effects of ice formation such as mechanical disruption of cell-to-cell relationships, concentration of dissolved solids and reduction of cell volume by intracellular water loss to growing extracellular ice.

Of course, vitrification requires the introduction of large amounts of so-called "antifreeze" chemicals: principally agents such as DMSO, formamide, and propylene glycol. These agents are good at forming glasses and prevent water from turning into ice at low temperatures. Unfortunately, concentrations of these agents high enough to inhibit freezing may be damaging in and of themselves. Much of Greg's report centered on his recent work in finding ways to minimize the toxicity of these cryoprotective mixtures. He was able to report that one particular mixture, a DMSO-formamide, propylene glycol, PVP solution was essentially nontoxic to the kidney cortex and yet was compatible with vitrification. Hopefully the next few months will see further progress and the recovery of the first viable vitrified kidney slices!

Paul Segall was next with an update on his hamster perfusion project. Paul reported numerous technical advances in preparation of the animals for washout including development of a new cannula pulling machine (not surprisingly hamsters have tiny vessels which require tiny custom-made cannulae) and the introduction of ketamine as an anesthetic. Paul showed a videotape documenting the survival of two hamsters for several hours following hypothermic blood washout at near the freezing point of water. Paul and BPRD Vice President Harry Waitz hope to make much more rapid progress now that better funding is available.

Fred Chamberlain III wrapped up the technical sessions with an excellent paper on deferred compensation schemes. Fred has spent a great deal of time thinking about ways in which people who put forth energy and effort early on in a cryonics (or other) enterprise can reap benefits in the future without jeopardizing the organization today. Fred presented two different scenarios for deferred compensation, one for nonprofit groups and one for profit groups. We hope to see a fuller exposition of Fred's ideas in this area in the pages of CRYONICS sometime in the future. I apologize for not doing a better job of summarizing Fred's talk, but there is no easy way to explain this idea simply in a paragraph or two and really do it justice.

That evening following the technical session we met again at the Timbercove for a banquet. I gave a brief introductory talk about cryonics developments in Florida and then turned the floor over to Saul Kent, featured speaker of the evening. Saul spoke at length about the early days of cryonics, taking as his theme the 20 years which have elapsed since publication of "The Prospect of Immortality." Saul's talk brought back memories for all of us. He ended with an affirmation of greater future personal participation in cryonics and an overview of some of the areas which he plans to devote himself to: raising money for research and generation of new promotional literature and approaches.

In the wee small hours of Monday morning the last few of us longwinded stragglers wandered back to our rooms for a few hours rest before heading for home or the sailing excursion on the Woodwind the following day.

I was lucky enough to be able to take in the Woodwind trip, so I spent

a sunny Monday lying around on the boat munching bread and fruit. Not that the trip was just a gravy run! We were able to watch Tahoe's two paddle wheelers race: the M.S. Dixie and the Tahoe Queen. We understand from just about everybody we talked to that the people who run the Tahoe Queen are not the nicest people in the world. The Queen, which is only a year old, was given good odds by the local casinos to win over the Dixie (which was built in the 1920's). All of us who have experienced the Dixie's hospitality (and a few who had experienced the Queen's lack of it) were rooting for the old girl to win! All of us were surprized when the Dixie not only won, but pulled out front immediately and promptly got yards and acres ahead of the younger Queen--and stayed there for the whole race!

After lunch and the race I promptly laid down on the deck and drifted off to a much deserved nap. Everyone laughed at me for wearing my floppy cap which looks like something out of Gilligan's Island. However, a couple of people with second degree sunburns the next day were NOT laughing. All I got was a slightly scorched nose.

I understand that the Chamberlains are going to do it again next year and that the program is supposed to reflect a broader line-up of technical subjects, in other words more aging/health and less cryonics. Ho hum, Oh well, if you MUST. But, this cryonics junkie enjoyed the meeting just as it was; an opportunity for a bunch of the really serious life extensionists (i.e., cryonicists) to get together and share some time in Paradise.

Next year's meeting is already scheduled for Memorial Day Weekend (appropriate isn't it?) and Fred and Linda are advising people to make PREPAID reservations NOW. This will prevent gouging and lack of available accommodations closer to the meeting. Remember, you can always CANCEL your reservations if you can't go, but it's hard to GET reservations at the last minute if you get to go. We should also point out for those of you on a budget that Motel Six has fixed rates nationwide and that they do accept reservations a year in advance! This year a room at Motel Six was going for $16.00/night versus $68.00/night at the Timbercove. Quite a savings.

So, that's it for my coverage of the Tahoe Festival. Stay tuned for the technical papers which will appear in CRYONICS over the next few months. I hope to see all of you next year at Lake Tahoe, and I especially hope to see some of the new people I met at this year's festival long before another year rolls by!

SCIENCE UPDATES by Thomas Donaldson

PUTTING CRYOPROTECTANT INTO ORGANS AND TAKING IT OUT AGAIN

One problem faced by anyone who wants to preserve organs (and brains) is that of how to remove the cryoprotectant afterwards without damaging the organs. The problem is that when we attempt to remove cryoprotectant by diluting it with a perfusate, we create a difference in osmotic pressure, causing the cells to swell up. This blocks off the vascular system. It causes spastic contraction of the muscles lining the arteriole walls, further blocking perfusion; the swelling also causes generalized mechanical damage. To successfully freeze (or vitrify) organs, we therefore need means to reduce

this swelling or to protect the cells from its effects (which include a relatively prolonged absence of oxygen due to lack of blood flow).

Several papers, already discussed in CRYONICS in short summaries, have just appeared in complete form in CRYOBIOLOGY. I shall report on them here.

The problem of swelling when we try to remove the cryoprotectant happens even if we don't freeze the organs, but only introduce the chemicals into them. Therefore, solving it is only a part of solving the general problem of cryopreservation. We can try several different approaches.

The first possible approach consists of more elaborate and measured methods for introducing and removing the cryoprotectant. If extremely elaborate methods were needed, this would obviously create difficulties for cryonic suspension, since most of our efforts are (relatively!) low budget affairs. A need to build a complex custom apparatus, although something cryonics facilities will certainly do, would also certainly use our scarce resources of manpower and money. I feel that the ideas in a paper by G.M. Collins and N.A. Halasz (CRYOBIOLOGY 21,1-5 (1984)) therefore merit some attention, because these two scientists have attempted to produce an apparatus which would be SIMPLE.

The exact mechanical design of their perfusion machine, given that we want to freeze whole bodies or heads, and they want to freeze rabbit kidneys, doesn't have any immediate obvious application. The idea is to use the degree of vascular resistance as measured and calculated to assess how rapidly to introduce or remove cryoprotectant. If the arterioles swell up, they provide a resistance to flow and prevent perfusion. This is vascular resistance. One idea Collins and Halasz provide, which seems quite significant to me, is their idea for measuring the vascular resistance. Other cryobiologists have attempted to measure this parameter by taking other measurements such as perfusion pressure and flow rate and applying empirical formulas. Collins and Halasz provide a simple circuit by which they can directly measure vascular resistance by comparing it with a fixed, known resistance. This method seems much simpler than others and should be simple to implement.

A second method for dealing with the problem of osmotic swelling consists of finding means to increase the ability of the cells to withstand the ischemia caused by the swelling. An article in the same issue of CRYOBIOLOGY (21, 13-19 (1984)) by J.H. Southard, F.O. Belzer, et al. bears upon this problem. I would like to point out that the intent of this article was to study methods of improving kidney survival during "high temperature" perfusions at 6-8 degrees C. A close reading of this article makes me think it also bears on frozen or vitreous preservation.

What Southard, Belzer, et al. have done consists of finding a way to increase the level of ATP in cells of perfused kidneys. (ATP (adenosine triphosphate) is the basic molecule our cells use to transfer energy. Oxidation of food molecules produces ATP rather than supporting our many biochemical reactions directly. When cellular ATP levels are low, cells have little energy available for metabolism.) They did this by adding several chemicals to their perfusate known to retard the breakdown of ATP, such as PO4, adenosine, and deoxycoformycin (an inhibitor of ATP breakdown). After five days of hypothermic perfusion at 6-8 degrees C, with their additional perfusates, they found that levels of ATP were about 2.5 times normal. Their exact results do not seem relevant to subzero organ preservation, but their method of increasing ATP levels may be applicable.

Finally, a third method of dealing with osmotic swelling consists of finding means to reduce or prevent the osmotic swelling itself. A very interesting paper by F. Pennickx, et al. at the University of Louvain in Belgium describes one means they found to do exactly that. Their method was to add sorbitol, a nonpenetrating sugar, to their perfusate and remove the perfusate

afterwards with high sodium, low potassium solutions. Their paper reports tests of this system on a very simple model red blood cells. Adding sorbitol completely counteracts the osmotic swelling otherwise caused by DMSO in this system. It does not effectively counter the swelling caused by glycerol, but does tend to reduce it a little.

All of these approaches and ideas merit a lot more work. Evidence exists suggesting that mechanical and vascular injury of freezing in whole organs may stem, to as yet unknown degree, from osmotic swelling and the failure of perfusion it causes (I.A. Jaconsen, et al. Cryobiology 12 (1975) 123-129). These papers make clear that many approaches exist to solve this fundamental problem.

VITRIFICATION RATHER THAN FREEZING

When some substances are cooled they do not crystallize but instead behave like glass, becoming more and more viscous until finally they respond so slowly that in ordinary terms they are virtually solid. For cryonicists, the interesting facts about such substances are, first, that mixtures of several cryoprotectants with water can show this glassy behavior, and second, that much of the damage of freezing comes not from low temperature as such but from damage due to the crystallization of water into ice. If we had some way of causing this water (or mixture of water and cryoprotectant) to vitrify rather than freeze, we might avoid many of the problems caused by freezing.

This is a simple, fundamental idea. To actually implement it in practice needs a great deal of work and a much more profound understanding both of vitrification and of the response of cells to the high levels of cryoprotectant needed to vitrify.

G. M. Fahy, et al. at the Red Cross Cryobiology Laboratory have just published a paper full of ideas and studies on how to vitrify organs for long-term preservation. They present both problems and many approaches to the solution of these problems.

First, all cryoprotectants are toxic to cells; although low temperature helps to minimize this toxicity, this problem happens even without freezing. If we attempt to vitrify an organ, we must expose it to concentrations of cryoprotectant much higher than those used for freezing.

Fahy, et al. suggest several different means of overcoming this problem. First, they can apply high pressure to the organ during cooling. A higher pressure lowers the concentration of cryoprotectant needed to reach a glassy, vitrified state. A pressure of 1,000 atmospheres will lower the concentration of DMSO needed to vitrify down from 44% to 38%, a nontrivial gain when even a per cent or two of DMSO may mean the difference between toxicity and recovery. Fahy, et al. report some some experiments with this idea in which they cooled concentrations of many different cryoprotectants, including DMSO, glycerol, and ethylene glycol and measured the concentrations needed to vitrify at 1 atmosphere and at 1,000 atmospheres.

A second method for overcoming the problem of high cryoprotectant concentration consists of adding a nonpenetrating cryoprotectant and making use of the fact that the high levels of protein available in an organ will affect the concentrations needed to vitrify. The water within the cells would vitrify at a lesser concentration due to intracellular protein, while the water outside the cells, because of the presence of the nonpenetrating cryoprotective agent, will also vitrify. Fahy, et al. also did experiments with this idea, using several different solutions of cryoprotectant and

slices of kidney tissue, and found that this method also will reduce the concentration of cryoprotectant

within the cells needed to vitrify. Their least toxic combination solution was a mixture of DMSO, acetamide and propylene glycol (they called DAP). The acetamide component in these authors "least toxic solution" acts specifically to neutralize the toxicity of DMSO. Fahy, et al. point out that dextrose will also act the same way.

If we use pressure as a means of lowering the concentration of cryoprotectant needed for vitrification we immediately find ourselves in another problem. That is, the pressure is also toxic. Fahy, et al. report on experiments with damage to rabbit kidney slices by high pressure. One highly favorable natural coincidence turns out to be the fact that glycerol and propylene glycol are both highly protective to kidney tissue against the damage of high pressure; they also report preliminary experiments in which their least toxic DAP solution was also protective.

These methods don't go all the way towards a solution, but they come sufficiently close for the authors to feel that the problem merits much more work. To vitrify, they must add to their 40% solution of DMSO, acetamide and propylene glycol an additional 6% of PVP (a nonpenetrating cryoprotectant). This causes toxic damage which they haven't yet overcome. However, they do present some preliminary studies of its nature with a view to working out counteracting methods; the toxic damage seems to happen because PVP may destabilize cell membranes. They also present a table of methods they've tried which don't work.

Once we have vitrified an organ we face another problem, that of finding a way to warm it up again without causing the glassy solution, which may be quite unstable, to freeze over into ice during rewarming. For practical reasons, we would like to have a means to warm organs without extensive apparatus. They present some evidence, involving studies of solutions of propylene glycol, that they will NOT have to pressurize the organ during heating in order to prevent it from crystallizing. Their suggestion is that a sufficiently high rate of heating will prevent crystallization, and that this rate may not be too high. Even if a rate as high as 1000 degrees C per minute is needed, microwave heating methods may provide it. Finally, of course, they might simply have to reapply pressure.

Many scientifically inclined cryobiologists may remember the first time that they heard of the experiments of Farrant (NATURE 205, 1284-1287 (1965)), who was one of the earliest cryobiologists to attempt vitrifying rather than freezing for cryoprotection. We all thought that such methods showed promise and regretted for a long time that no one had followed up on them. Farrant only did a few experiments and seems not to have pursued the problem very far. Certainly his work alone would not be sufficient to freeze organs. It is very important indeed that someone has finally started to study this approach in earnest.

BRAIN CELL PRESERVATION AFTER FREEZING

Despite (and perhaps because) the subject is so important, papers on freezing brain cells rarely get published. Of course, cryonicists will understand that freezing brains counts as the single most interesting and important topic in cryobiology; however we can't expect that most scientists will study interesting and important things.

A recent paper in Cryoletters (5 (1984) 33-46) by G.M. Fahy, T. Takahashi, and A.M. Crane, at the Red Cross Cryobiology Laboratory, adds

one more to the short list of papers reporting studies of the response of brain cells to freezing. For cryonicists this paper is interesting because it comes up with

some particularly cogent data: namely that even without cryoprotectant, frozen brain tissue may retain a high degree of structure. So far as we can use survival of structure as a guide to survival of personality, it therefore adds evidence to the thesis that our memories and personalities will survive freezing.

The experiments of Fahy, et al. were simple. They studied brains of rabbits and rats, frozen with several different cryoprotectants, including glycerol, DMSO, propylene glycol, and methanol. The major outcome of their study was the good preservation of brains cells evident with light microscopy. They found that glycerol would penetrate well into rabbit brains, but not nearly so well into rat brains. The two other cryoprotectants, propylene glycol and methanol did permeate well into the rat brains. Furthermore, cells from these frozen brains, once we could discount the damage caused by sectioning and preparation for the microscope, seemed quite well preserved in cell structure and location. Fahy et al. point out, in fact, that slides of cryoprotected rabbit brains could not be distinguished from slides of control, unfrozen rabbit brains. Even better, when they took slides of the rat brains, even without cryoprotectant the cell structure and anatomy appeared reasonably well preserved.

Our major problem in judging survival of memory and personality consists of the fact that we don't know how our memories are encoded. One of many possibilities is that memory is encoded through the existence of cellular connections; although right now this possibility seems less and less likely as more research on the nature of memory is done. The implication of this paper by Fahy, et al. is that the gross structure of brain tissue undergoes very little disruption because of freezing, especially if cryoprotectant is present. All the existing hypotheses of memory storage would therefore conclude that memory also survives. True, someone determined to create an argument to the contrary could produce ad hoc hypotheses to reach their desired conclusion.
However, no amount of experimentation can influence such a cast of mind.

A close reading of this paper reveals another point. The authors perfused their animal's brains with cryoprotectant while the brains were still in the animals, and removed the brains afterwards. In fact, they set up a perfusion circuit by opening the chest of the animal in much the same way as we get access to circulation for cryonic suspension. Their work therefore suggests that brain cell preservation in contemporary, state-of-the-art cryonic suspension may resemble that in their animal experiments. Furthermore, the paper has something very important to say about possible cryoprotectants for suspension. It points out that not all species respond to cryoprotectants in the same way, since brains of rabbits and rats showed quite different abilities to take up glycerol.
As cryonicists we therefore need work on primates to test that glycerol really is the best cryoprotectant for human brains.

Finally, one rather fascinating side issue concerns the authors' observation that rat brain tissue frozen without cryoprotectant actually seemed well preserved, at least from a gross (light microscopic) standpoint. I feel that this is important because not all human freezings can take place under the best of conditions. Cryoprotectant may not be available or someone may have been accidentally or deliberately frozen without cryoprotectants. It's therefore very important for us to know what the consequences of that may be for brain cells, and even more important to

know that we may actually still have a high degree of survival. Of course no one would recommend straight freezing as an optimal procedure!

EVALUATION OF HEAT EXCHANGE MEDIA FOR USE IN HUMAN CRYONIC SUSPENSIONS

by Michael Darwin and Hugh Hixon

The current procedure employed for cooling both whole-body and neurosuspension patients from 0 degrees centigrade to the temperature of dry ice (-79 degrees centigrade) is to immerse the patient in an isopropyl alcohol (isopropanol) bath and gradually add dry ice to achieve a cooling rate of approximately 2 degrees centigrade per hour. Isopropanol has been used as a heat exchange medium because it has an appropriately low freezing point, is low in cost and presents no insurmountable handling difficulties or hazards. The original choice of isopropanol as a heat exchange medium was an obvious one: it is commonly used by clinicians and cryobiological researchers who wanted much the same properties that were desired by cryonicists. However, as discussed below, isopropanol has a number of serious disadvantages for use in human cryostasis operations. The problems have proved to be serious ones and have led the authors to an extensive search for alternative heat exchange media, as discussed below.

*** TYPIST'S NOTE: FIGURE ONE WAS A GRAPH OF TEMPERATURE IN DEGREES CENTIGRADE VS. TIME IN HOURS, MEASURED FOR RECTAL, SURFACE, AND AIR TEMPERATURES. FIGURE TWO WAS A GRAPH OF SIMILAR COMPOSITION. ***

FIGURE I: Passive air cooling curve of a 5 kilo preserved cat.

FIGURE II: Convection augmented cooling curve of a 5.0 kilo preserved cat.

THE NEED FOR LIQUID HEAT EXCHANGE MEDIA

The most desirable alternative to the use of isopropanol would be to abandon the use of liquid heat exchange media altogether and return to gas cooling, of either a passive or forced variety as was employed early on in cryonics procedures(1,2). In the event very slow cooling rates can be demonstrated to be desirable, the use of gaseous heat exchange may be practical. As is shown in Figure I, gas is a relatively poor heat exchange medium. Even the addition of a powerful convective system as illustrated by Figure II does not result in rates of cooling comparable to

*** TYPIST'S NOTE: ORIGINALLY FIGURE THREE WAS A GRAPH OF TEMPERATURE IN DEGREES CENTIGRADE VS. TIME IN HOURS, WITH BOTH RECTAL AND SURFACE TEMPERATURE LISTED. ***

Figure III: cooling curve obtained when a 5.0 kilo preserved test cat was placed in an isopropanol bath to which dry ice was added at a controlled rate.

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those which may be achieved with liquid cooling (Figure III). Current cryobiological consensus is that it is desirable to avoid long periods of exposure to high cryoprotectant-solute concentrations at high subfreezing temperatures. Once freezing begins, it is desirable to reduce the temperature as rapidly as possible without inflicting injury on a gross level such as fracturing due to differential contraction of materials or expansion/shell deformation effects (3) (4) (5). Only liquid heat exchange media will provide efficient enough heat removal to meet this requirement.

DISADVANTAGES OF ISOPROPANOL

Aside from the advantages of appropriate freezing point, low cost and ready availability, isopropanol has a number of serious disadvantages. It is flammable and thus it presents a serious hazard for storage. It is volatile at room temperature with a boiling point of 82.4 degrees centigrade and a flashpoint of 12 degrees centigrade. Direct addition of dry ice to the isopropanol, as well as pumping operations, also expose personnel to wetting of clothing with a flammable liquid. All of these are serious safety risks to staff involved in storage or use of the material.

A more immediate concern is the damage isopropanol does to suspension patients exposed to it. In the past, Trans Time, the only cryonics company which has employed alcohol cooling, has attempted to protect patients from

exposure to isopropanol by wrapping them in fluid-tight bags made of ethylene vinyl acetate (EVA), a plastic with good flex rating down to dry ice temperature. Unfortunately, this maneuver has consistently failed to protect patients from exposure to isopropanol for a number of reasons. First, it is difficult to load an adult human with sensors, monitoring wires and associated paraphernalia into a large plastic bag without causing small holes in the bag. The bags themselves are probably subject to puncture during manufacturing, packing and shipping and there is some question as to the integrity of their electrically welded seams. Secondly, despite the excellent thermal rating given EVA in the literature, in practice it tends to become brittle as it approaches dry ice temperature, losing its room temperature properties which are much like those of heavy polyethylene, and acquiring a stiffness and brittleness more characteristic of acetate or polypropylene films. The net effect of these deficiencies has been consistent leakage of the bags with consequent exposure of the patient to isopropanol.

Exposure of patients to isopropanol at very low temperatures is a serious problem. Even at -79 degrees centigrade the liquid isopropanol can readily move into tissue and dissolve ice, a process used as a histological preparative procedure and known as freeze-substitution (6). This is a devastating effect and may be likened in terms of its biological

consequences to embalming. Alcohols, including isopropanol, are also excellent solvents for lipids, even at dry ice temperature, and can be expected to elute lipids from cells exposed to them. While short-term exposure of the surface of a patient's body to isopropanol is not likely to result in serious damage, long-term exposure is almost certain to. It should also be pointed out that many body membranes that will be exposed if the patient is immersed in isopropanol are quite thin or otherwise unprotected and would be almost immediately damaged. Examples of these kinds of structures would be the cornea of the eye, the tympanic membrane (ear drum), the oral and nasal mucosa, the entire pulmonary gas exchange surface and of course any surgical wounds and the organs beneath them. Prolonged exposure of the entire body to isopropanol at or above dry ice temperature would result in serious freeze substitution and significant penetration of the isopropanol into body tissues.

Because of the hazards of isopropanol to both personnel and patients a search was undertaken for a more appropriate heat exchange medium.

REQUIREMENTS FOR A HEAT EXCHANGE LIQUID

The first step in the search for an improved heat exchange medium was to establish a set of "ideal" criteria against which to judge candidate compounds. These criteria were:

Nonflammability
Nontoxicity
Boiling point above 100 C
Pour point below -79 C
Freezing point below -100 C
Low volatility at room temperature
Clarity over full range of temperature use
Indefinite shelf life
Chemical inertness with common solvents and cryoprotective agents
Insolubility in water
Affordability

An initial evaluation in the literature of a wide variety of materials revealed three classes of compounds with general properties roughly suited to our application: fluorocarbon liquids, the Freons(TM)* and the organic silicone polymers.

Despite excellent physical and biological properties, consideration of the fluorocarbon liquids, such as 3M's FX-80 was not a serious possibility due to extreme cost: $22.00/lb.

Of the Freons, two compounds were selected for evaluation on the basis of their freezing points, boiling points and toxicity: Freon MF and Freon TA. Samples of both compounds were obtained from the McKesson Chemical Company in Santa Ana, California and evaluations conducted. Both compounds exhibited freezing points approximately 10 degrees centigrade below the rating given them, and boiling points within 1 to 2 degrees centigrade of where they were rated. Freon MF had a rated freezing point of -111.0 degrees centigrade and a boiling point of 23.8 degrees centigrade. The literature for the material indicated a relatively flat viscosity curve between room temperature and -80 degrees centigrade and tests showed that it tolerated direct addition of dry ice in much the same fashion as isopropanol.

Unfortunately, Freon MF was extremely volatile at 20 degrees centigrade

and boiled vigorously at 40 degrees centigrade. Freon MF was also an excellent defatting agent and quickly caused irreversible respiratory distress in mice exposed briefly to aerosols of it. Due to the low boiling point of Freon MF and its good lipid solubility we eliminated it from consideration as a suitable heat exchange medium for human cryonic suspensions.

The other Freon we evaluated, Freon TA, is an azeotrope (intermolecular compound) of Freon TF and acetone which combines the desirable safety characteristics of Freon TF with the low freezing point of acetone. Freon TA was extensively evaluated and found to perform well as a heat exchange medium. Freon TA was about as volatile as acetone at room temperature but was not flammable even with exposure to direct flame from a propane torch. Water solubility in Freon TA at 25 degrees centrigrade is less than 0.15% (7). Toxicity of the material is reportedly less than for isopropanol. Unfortunately, Freon TA presents a number of serious drawbacks as well as advantages. The azeotroping is readily reversible with water and results in liberation of acetone from the compound which in turns presents a serious fire and explosion hazard. Exposure of the compound to open flame or high temperature heating elements (such as electric space heaters) results in decomposition of the compound into hydrofluoric acid which presents a very serious corrosion and health hazard. The compound is also a good defatting agent and was seriously injurious to the lungs and mucous membranes of mice subjected to environmental saturation with the agent. In spite of the low cost of the compound per kilo, its disadvantages caused us to set this agent aside and continue our search for a better heat exchange medium amongst the silicone fluids.

SILICONE COMPOUNDS

The organic silicone polymers are manufactured almost exclusively by the Dow Corning Company of Midland, Michigan. Of the silicone fluids offered by Dow Corning, three agents appeared of interest to us: DC 200 fluid (0.65 and 5.0 centistokes)(polydimethylsiloxanes), DC 225 cosmetic fluid (9.5 centistokes)(a dimethicone), and DC 345 cosmetic fluid (4-8 centistokes)(a polydimethylcyclo-

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*FREON is E.I. Du Pont De Nemours' registered trademark for its fluorocarbon compounds.

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siloxane). These compounds are used extensively in the cosmetics industry as surface spreading agents to increase the effectiveness of other ingredients and a vapor barrier to retard water loss or prevent its penetration (the latter as in hair-sprays) under high humidity conditions. Most hand lotions employ silicone compounds to protect the skin against water loss and provide lubricity and "silkiness." Samples of each of these compounds were obtained from Dow and evaluated. Excellent toxicity data was available from Dow Corning on all three of these compounds (8) (9) (10) (11) (l2) (13) (14). The toxicity of all these materials was very low with the acute oral LD50 being in excess of 35g/kg. Chronic feeding to rats (one year) and rabbits (eight months) at one percent of their daily diet produced no adverse effects. All fluids are nonirritating to the skin, even with prolonged and repeated contact. Saturated aerosol inhalation tests produced no adverse effects to albino rabbits receiving 130 exposures of 15 seconds duration over a period of 90 days. Dow Corning reports

TABLE I

PROPERTIES OF SELECTED HEAT EXCHANGE FLUIDS

AGENT:	Isopropanol	Dow Corning fluids 200 .65 cs	200 5.0 cs	225 10 cs	345 4-8 cs	Freon TA
Color at 25 C	clear	clear	clear	clear	clear	clear
Color at -79 C	hazy	hazy	clear	white	white	clear
Color at -60 C	hazy	hazy	clear	hazy	hazy	clear
Fluidity at -79 C	syrupy	fluid	fluid	solid	solid	fluid
Fluidity at -60 C	syrupy	fluid	fluid	syrupy	syrupy	fluid
Melting Point	-89.5	N/A	N/A	-63.5	-60.5	-80
Specific Heat cal/gm- C	.44	.41	.41	.360	N/A	.305
Specific Gravity g/ml	.786	.761	.920	.94	.96	1.406
Odor (0-5)	5	4	0	0	0	5
Flash Point C	12	26	135	163	82	none
Toxicity	moderate	none	none	none	none	low
H O Solubility	soluble	insol	insol	insol	insol	low
Shelf Life	indef.	12 mo.	indef.	indef.	indef.	indef.
Volatility mg/cm /hr @25 C	12	20	0	0	0.7	26
Price $/lb 55 gal.	0.47	21.00	6.07	6.55	6.55	1.17

that some transient conjunctival irritation is noted if the material is introduced into the eye. However, our toxicity evaluation (described below) did not disclose this effect.

We evaluated these agents for freezing point, melting point, volatility, flammability, and performance at low temperatures. Results of this evaluation appear in the table above, along with other relevant data. Of the silicone fluids evaluated, the DC 200(0.65cs) fluid had the most desirable properties with respect to viscosity at -79 degrees centigrade. However this material was extremely volatile and highly flammable. Even a spark from a flint was sufficient to produce a raging fire which was extraordinarily difficult to extinguish. Despite the excellent low temperature performance characteristics of this material it was deemed unsuitable due to its extreme flammability. This left us with the DC 200(5cs) fluid and DC 225 and 345 cosmetic fluids. Both of the cosmetic

fluids performed similarly when cooled to dry ice temperature. Both fluids readily supercooled but eventually underwent a phase change (crystallized) at -79 degrees centigrade after prolonged exposure to this temperature. The 225 fluid froze to a soft, waxy solid of about the consistency of vegetable shortening. The 345 fluid was by comparison firmly frozen at -79 degrees centigrade and had the consistency of paraffin or beeswax at room temperature. At -60 degrees centigrade both fluids exhibited a viscosity close to that of heavy motor oil at room temperature, but could be considered

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*** TYPIST'S NOTE: FIGURE FOUR WAS A GRAPH ENTITLED "VISCOSITY TEMPERATURE CHARGE FOR DOW CORNING 200 FLUID," AND COMPARED TEMPERATURE IN DEGREES C. AND KINEMATIC VISCOSITY IN CENTISTOKES. ***

FIGURE IV: Viscosity vs. temperature for various DC silicone fluids as contrasted with 10 W 30 motor oil.

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pumpable. However, because these materials froze at dry ice temperature, they were eliminated from consideration. The DC 200(5cs) fluid did not become cloudy or freeze even after extended exposure to dry ice temperature. Efforts to determine a freezing point for this material were unsuccessful: moderate to rapid cooling to liquid nitrogen temperature resulted in vitrification of the material. Attempts were made without success to induce freezing of the agent by seeding at -79 degrees centigrade with a variety of debris as well as by seeding with crystals of frozen DC 200(12,500cs) fluid.

The viscosity of the DC 200(5cs) fluid reported in the literature (Figure IV) (15) as extrapolated to -79 degrees centigrade was 350 cs or roughly comparable to olive oil at room temperature. The fluid exhibited no haziness or other loss of clarity on cooling and could easily be pumped at dry ice temperature.

To evaluate the low-temperature viscosity of DC 200(5cs), we employed a cup viscosimeter of the type used for thinning paint. Several other liquids were run for comparison, and the results are tabulated in Table II below.

TABLE II
CUP VISCOSITY OF SEVERAL HEAT EXCHANGE FLUIDS

Fluid	Temperature (C)	Viscosity (seconds)
Water	23	14.2
Isopropanol	21.5	12.6
	-71.5	27.5
DC 200(5cs)	24.0	13.2
	-76.5	17.3

Liquid is allowed to run out of a cup through a hole in its bottom. The time taken for a standard volume of liquid to run through a hole of set size is a (non-linear) measure of the liquid's viscosity. Times are the average of five determinations.

As can be seen from the times, DC 200(5cs) is only slightly more viscous at dry ice temperatures than water is at room temperature, and much less viscous than isopropanol at dry ice temperature.

TOXICITY EVALUATION

Because extensive patient exposure to the heat exchange media is likely, we thought it advisable to evaluate the toxicity of the DC 200(5cs) fluid in-house. Also, it should be pointed out that Dow Corning's toxicity evaluations were not designed to evaluate the fluid under the conditions likely to be encountered in human cryonic suspension operations: prolonged immersion, massive pulmonary exposure and wound exposure.

We sought to evaluate the effects of prolonged immersion in the fluid by thoroughly wetting albino (white) mice in the fluid and then placing them in

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1/4" of the liquid with a nebulizer present at 31 degrees centigrade for five hours. This technique, hereinafter referred to as "environmental saturation" prevents the animals from removing the fluid from their skin and provides an atmosphere which is saturated with the fluid as well. During environmental saturation the animals also ingest large amounts of the material in an effort to remove it from their fur and skin. The eyes, ears and mucous membranes remain constantly in contact with the fluid during the course of the exposure.

Following environmental saturation animals were returned to clean bedding and allowed to remove the fluid from their coats over the course of the next 3-4 days. Owing to the poor water solubility of the fluid it is difficult for the animals to remove it from their coats, thus extending the time of cutaneous exposure to at least three days. This test was undertaken twice, with two groups of five animals. One animal from each group was sacrificed immediately post exposure and autopsied to evaluate any acute effects such as pulmonary inflammation or nasal or pharyngeal mucosal irritation. No evidence of acute toxic or irritable effects were seen in any of the autopsied animals. The stomachs of both animals were noted to be distended with the silicone fluid.

The remaining animals were followed for a period of 27 days following environmental saturation exposure. During the first 72 hours post exposure ronchi and a nonproductive cough were noted in four of the eight animals. Both of these symptoms disappeared within a few days and the animals exhibited no other sign of toxicity as a result of saturation exposure. At 27 days the animals were sacrificed and autopsied. The autopsies were unremarkable with no significant abnormalities noted and no evidence of irritation or scarring present.

A second test was undertaken to determine the toxicity of DC 200(5cs) fluid by injecting mice and rabbits intraperitoneally with the fluid in doses of 2.5 and 5.0 cc/kg. The fluid was sterilized on delivery by passing it through a .2 micron Millex filter placed between the syringe and the needle. Mice were given the fluid with B-D plastic tuberculin syringes, a technique which was complicated by rapid dissolution of the

silicone grease used to lubricate the plunger of the plastic syringe by the DC 200(5cs) fluid. Because of the rapid loss of plunger lubrication and "sticking" of the plunger in the barrel of the syringe we switched to glass syringes for administration of the fluid to rabbits.

Two groups, of five mice each, were given DC 200(5cs) at 2.5 cc/kg and 5.0 cc/kg respectively. Two of the five animals in the 2.5 cc/kg group died 72 hours after administration of the material. Autopsy failed to disclose the cause of death: there was no sign of peritoneal inflammation and hearts, lungs, kidneys and livers were unremarkable on gross examination. The surviving three animals continued to do well and were sacrificed and autopsied 28 days following administration of the material. No abnormalities were noted in any of these animals.

Administration to mice of DC 200(5cs) at 5.0 cc/kg resulted in a profound reduction of food and water intake and death of four of the five animals within 72 hours. Autopsy of the animals disclosed no obvious cause of death.

The results of intraperitoneal administration of relatively large doses of DC 200(5cs) fluid to mice prompted us to evaluate the effects of this material on larger animals. To this end, we administered 2.5 cc/kg intraperitoneally to three New Zealand White rabbits. Twenty-four hours after administration one of the animals was sacrificed and examined for signs of peritoneal inflammation or other toxic effects. The autopsy was unremarkable. The two remaining animals continued to do well and were sacrificed and autopsied 60 days following administration of the fluid. Autopsies of these animals disclosed no apparent chronic effects of DC 200(5cs) administration. Numerous small parenchymal

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hemorrhages in the lungs of both the experimental animals were noted. However, these were believed due to exposure to an odor control agent used in the dropping pans of the cages (control animals also exhibited these lesions at autopsy). No hepatomegaly, renal edema, or other signs of toxicity were noted. The peritoneum and mesentery of both animals was grossly normal and free of any signs of adhesion or scarring.

A second group of two rabbits was given DC 200(5cs) at 5.0 cc/kg. Immediately following administration of the fluid the animals became oliguric, and ceased all water and food intake. No disturbance in behavior was noted and the animals ambulated and behaved normally. Water and food consumption gradually returned to normal over the following 72 hours and remained at normal levels until the animals were sacrificed 12 days following administration of the fluid. Autopsies of these animals disclosed no evidence of acute irritation and the appearance of the abdominal and thoracic viscera were normal.

Application of the fluid to the corneas of five New Zealand White rabbits produced no obvious irritation, and examination of the animal's eyes with a fluorescein stain under ultraviolet light revealed no sign of irritation at either 30 minutes or 24 hours following application.

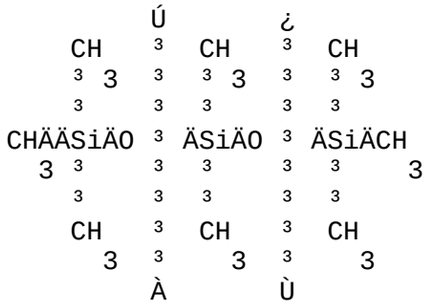
REUSE

Because of the significant expense of this material we have conducted a number of tests to determine if it can be reused. We have found, not surprisingly, that DC 200 fluid tends to accumulate water with prolonged periods of exposure to air when the material is at subzero temperatures. The DC 200 fluid may be easily dehydrated by passing it over anhydrous calcium chloride, or if the contamination is gross and the fluid is at room temperature, by pumping it through a diesel water filter of the type used in automobiles or through a roll of household paper towels. The fluid

passes easily through most common filter elements and may be sterilized following prefiltration for dirt removal (clarification) by passing it through a 0.2 micron filter. Removal of lipids and dissolved organics should be achievable via activated carbon filtration.

SUMMARY AND CONCLUSIONS

On the basis of all of the above considerations, we feel that Dow Corning 200 fluid (5 centistokes) is probably the best choice of the agents we evaluated for use as a heat exchange medium in human cryonic suspension operations. The chemical formula of the DC 200 fluid family is:



While the material does not have a freezing point as low as we would like, it does remain quite fluid down to -79 degrees centigrade: a temperature sufficiently below the freezing point of most commonly used cryoprotectant-water mixtures to be of real use. The heaviest demands are placed on the heat exchange medium in the 0 degrees to -40 degrees centigrade range--a temperature at which the DC 200(5cs) fluid is very fluid and easily pumped. The low

toxicity and indefinite shelf life also help to put this material ahead of other candidates examined. The one serious drawback to use of this material is its price: $6.07/lb or nearly $2,800 for the roughly 55 gallons of the material needed to accommodate the average whole-body patient. A mitigating aspect to the relatively high cost of the material is the fact that it can be reused a number of times making the material more of a capital expenditure than a "disposable."

We have evaluated a variety of compounds for use as a heat exchange medium in human cryonic suspensions. Most were rejected due to undesirable properties with respect to flammability and/or toxicity. One fluid, a low molecular weight polydimethylsiloxane, Dow Corning 200 (5 centistoke) fluid was selected as being closest to the "ideal" fluid specified at the start of this study. We have selected DC 200(5cs) fluid as the most logical replacement candidate for isopropanol, the heat exchange medium currently in use by cryonics organizations.

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