

CRYONICS

NOVEMBER 1984

ISSUE # 52

CONTENTS

Editorial Matters.....	page 1
Turkey Roast Time!.....	page 2
Trans Time Sells Videotape.....	page 2
Frosty Thermometer.....	page 3
Two More Total Body Washouts.....	page 3
ALCOR Paperwork Ready.....	page 4
Cemetery Plots Needed.....	page 5
New Autopsy Legislation.....	page 5
Science Updates.....	Page 6
BACS Updates.....	page 7
Histological Study of a Temporarily Cryopreserved Human.....	page 13
Letters.....	page 32
Prepaid Suspensions and Ethics.....	page 33
ALCOR Meeting Schedule.....	page 37

CRYONICS is the newsletter of the ALCOR Life Extension Foundation, Inc. Michael Darwin (Federowicz) and Hugh Hixon, Editors. Published monthly. Individual subscriptions: \$15.00 per year in the U.S., Canada, and Mexico; \$30.00 per year all others. Group rates available upon request. Please address all editorial correspondence to ALCOR, 4030 North Palm #304, Fullerton, CA 92635 or phone (714) 738-5569. The price of back issues is \$2.00 each in the U.S., Canada, and Mexico, and \$2.50 for all others.

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EDITORIAL MATTERS

Sprucing Up

One of the complaints we've heard from time to time about CRYONICS is that we have "huge, unbroken walls of text"

which are intimidating and difficult to read. We acknowledge this a problem and we've tried to break things up a bit with illustrations and greater spacing between articles. Still, the problem persists. For several reasons, going to double columns of text is not practical for us (particularly with the word processing program we have). We have also tried to bring you a lot of material and still do it in an economical fashion. Starting with the December issue we're going to experiment with spaces between paragraphs and the use of subheadings to break things up even further. We mention these changes so that contributors who send us camera-ready material can include subheadings in their pieces and so that our "cover-to-cover" readers will realize that we're not trying to "skimp" on copy.

#### The Cost

A count of the October CRYONICS indicates that we will lose roughly 6-7 lines of material per page of text edited this way, or about one page for every 8 pages of text. The one of us (HH) most concerned with page formatting does not like the page loss, the loss of flexibility in formatting pages, or, if we chose to maintain the text output, the increased production costs. About the only advantage to us is personal--the change means 4 pages of text that we do not have to come up with every month. It should be noted that HH is a voracious reader, and does not consider good paragraph format "unbroken walls of text." On the other hand, this magazine takes considerable effort to put out, and we like it to be readable, and read, by the largest number of people. Comments please!

#### The Co\$t

As noted above, these changes will cost money. Frankly, not a lot, but then every little bit hurts. At this time CRYONICS is costing about \$300 per month to put out--not including time (which is all volunteer). We should have raised our subscription prices long ago. We know of no comparable newsletter of the size and quality of CRYONICS which is available for \$15 per year! We have held off raising our subscription price because we know that many of our readers would really feel the pinch, and many new readers would be discouraged from subscribing. In large measure we've been able to hold the subscription cost down because of the generosity of a few folks who've given (repeatedly) directed donations aimed at allowing us to continue publishing. As the year ends, the question looms in our minds: will be able to afford to continue offering the kind of publication we have in the past during the coming year?

#### It's Up To You

To a great extent that depends on you. Not only CRYONICS, but all of

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(2)

ALCOR's activities are funded by voluntary giving. How much we are able to achieve depends on the resources we have available. Last year, we received over \$15,000 in donations. This money has fueled the research and educational activities you've seen reported in our pages. As the year draws to a close and tax time moves ever closer, we urge you to think about us. If you're among

the folks who make stiff, involuntary contributions to the IRS, you might consider diverting a little of the money our way. We're working hard to save your life, and we need all the help we can get.

Think about it. December 31st is just a few days away!

#### IT'S ALCOR TURKEY ROAST TIME AGAIN!

Another year has evaporated and once again it's time for the ALCOR Turkey Roast. For those who aren't familiar with this intellectual, social and culinary feast, we hasten to point out that the only ticket required for entry is a covered dish and a disposition to friendly conversation. We aren't sure exactly how long we've been having Turkey Roasts, but we are sure they've turned into a real ALCOR tradition. It's a tradition worth sharing, so grab a friend, some food and drink and be there.

For information on what to bring (so we don't end up with thirty desserts or thirty boxes of soda crackers!) give Maureen Genteman a call at (213) 392-2137. (Vegetarians should call Mike Darwin at (714) 990-6551) The Turkey Roast will be held at the lovely and spacious home of Marce Johnson in Huntington Beach.

Directions to Marce's can be found in the meeting Schedule on page 37 of this issue. So, let's see you there on Sunday, December 2nd at 1:00 PM!

#### TRANS TIME'S FINANCIAL SITUATION IMPROVES

We understand from Jack Zinn that Trans Time is in a more secure position now as a result of the sale of videotape to the Australian National TV Network for \$5,000. The eight minute long tape was sold for exclusive use in Australia. Trans Time has been troubled by sluggish cash flow recently, and this income will help to erase some of the negative balance that's been accumulating.

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(3)

#### FROSTY THERMOMETER

Frosty Thermometer is hung up at about the half way mark. We need at least another \$2,000 to get the vault and trailer outfitted and ready to accept the patient dewar. The trailer is in our hands now and the vault is waiting to be picked up in Fontana, CA. We need to quickly raise at least another \$1,000 for water containers and a framework to lower the dewar into the vault with. We also need to pay for forklift rent and for liquid nitrogen expenses associated with temporarily

transferring patients to a backup dewar while the A-2542 (primary dewar) is being loaded into the vault. Don't desert us now--we're almost there!

#### TWO MORE TOTAL BODY WASHOUTS COMPLETED

Since we reported on our success with Star in the September issue, we have undertaken two more total body washouts, both of these with four hours of asanguineous (bloodless) perfusion at 4 degrees centigrade, again using a slightly modified version of the base perfusate designed to be employed by ALCOR in human suspensions.

The first of these two experiments ended in outstanding success with long term survival of the animals. "Enkidu" (pronounced "Inky-do", named after the friend Gilgamesh sought to bring back from hell in "The Epic of Gilgamesh") is a good example of what dedication can do. "Inky", as he came to be called, required nearly a week of around the clock intensive care nursing. He did not eat his first solid food for nearly a week, and he required constant turning, suctioning, medicating and high quality supportive care day and night. Unfortunately, Jerry Leaf and Mike Darwin had to be away in the days following the experiment (they were attending the Society for Cryobiology meeting in La Jolla) and that left the job of nursing Inky back to health to Anna Tyeb and Hugh Hixon. Most of that difficult job fell to Anna, who virtually lived at the lab for a week--largely without relief--struggling to bring Inky safely back from the "dead." Indeed, it was Anna's suggestion that she (as well as the rest of us) had "gone through hell to bring him back to life" which suggested his name.

The photos on the cover show Anna with a fully recovered Inky behaving in his usual, uncontrollable way. One thing which Inky definitely demonstrated is the preservation of memory and personality after cooling to 4 degrees centigrade. Inky was unmanageable and "difficult" before the experiment, and this behavior was present after his recovery as well.

Four weeks after the experiment, Inky was sacrificed and subjected to fixative perfusion and a careful postmortem examination (including tissue histology) to check for any lingering effects of the four hours of cold asanguineous perfusion. Gross examination revealed no sign of injury. Histological examination will be completed in a month or two.

The third experiment was conducted on the weekend of the 29th of September and was also a 4 hour washout/perfusion, this time with cooling to 1 degree centigrade. After the experiment was underway, we realized that we had a very

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(4)

sick animal on our hands (apparently a viral intestinal infection). The intestinal infection, complicated by washout and anticoagulation degenerated into intestinal bleeding almost immediately post operatively and the animal died 12 hours after rewarming with only slight recovery of consciousness. We also noted some other problems (probably unrelated to the infection) with the pancreas and the dura (the dura mater is a tough membrane that covers the brain) which may have been a result of perfusion at so low a temperature. We are taking steps to try and avoid these problems in the future.

None of the animals demonstrated any sign of pulmonary edema and the necropsy performed on animal #3 showed the lungs to be in excellent condition. In the past, pulmonary edema has been a major stumbling block to recovery of animals after asanguineous perfusion.

We have 3 to 4 more experiments in this series planned, and we hope to have the work wrapped up by March or April of 1985.

As an aside to those who've asked, Star, our first TBW survivor has found a home with a family in the San Diego area and is reportedly doing very well. Star was a very special beast to all of us, and it gives everyone here at ALCOR great pleasure to know that his good natured gentleness is making other people happy.

ALCOR PAPERWORK: ON SCHEDULE  
AND READY TO GO!

The new ALCOR paperwork, which employs an Agreement or contract as the core document, is now essentially complete. While there will no doubt be much fine tuning in the months ahead, the basic documents are now ready for execution, and

indeed we have already executed three new contracts. If you are signed up using the "old" ALCOR paperwork, then you will need to execute a contract. You needn't fear, this is a simple exercise and requires only a few minutes time in most cases to answer the questions asked in the contract and to sign it. You only need two people (at least in California) to witness the Agreement and the Agreement is written in simple language which is easily understood. We will be notifying you over the next six months to a year of when you need to execute the Agreement. We are going to go down the list slowly, so that we can handle small groups of people and take time to explain things to them properly.

#### Avoid the Rush

Anyone who wishes to execute a contract right away should simply let us know and we'll get you a copy promptly. We are now in the process of writing instructions or "documentation" for the new paperwork and we anticipate we'll have that available in about six months. This documentation will provide step by step instructions and explanations so that people living distant from ALCOR can sign themselves up without an ALCOR representative walking them through the procedure.

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(5)

#### Major Changes

There are a number of major changes in the structure of the arrangements we offer. The contract, and the model Will form which accompanies it, now automatically provide for conversion to chemical or other preservation techniques (morphostasis) as a last ditch effort should funding for continued cryogenic care become inadequate.

The contract also defines the rights and duties of the patient as well

as the rights and duties of ALCOR. Items such as Emergency Responsibility, Remote Standby and other services are spelled out, as well as the terms under which they are available.

The ancillary paperwork such as the Relative's Affidavit has been broadened in scope and better provides for cooperation and noninterference on the part of next of kin.

The new paperwork package includes both a model Will and a model Durable Power of Attorney. In the near future we hope to add a model Limited Special Power of Attorney. Power of Attorney is something every cryonicist should consider providing for--either by appointing ALCOR, or some other trusted individual or organization, to manage their affairs should they become incapacitated. Otherwise, hostile, greedy, or indifferent relatives can expend resources set aside for suspension, move you out of the reach of cryonics services or otherwise frustrate your cryonics organization's attempt to suspend you should the need arise.

While ALCOR does not require it, you may request and execute the new Relative's and other ancillary affidavits to provide additional protection for yourself. All we will be requiring in this current update is execution of a contract.

#### CEMETERY PLOTS NEEDED

In order to provide for secure storage of our "remains" should simple preservation techniques (such as morphostasis) become necessary, ALCOR is interested in acquiring cemetery plots in the Southern California area. In fact, we are interested in plots anywhere within reasonable striking distance of Los Angeles. If any of our members have cemetery plots which they now (hopefully) have no use for, please consider donating them to ALCOR. Such donations which help to provide some additional security and have the advantage of being fully tax deductible as well.

#### NEW LEGISLATION LIMITING AUTOPSIES PASSES

A rather peculiar bill prohibiting voluntary autopsies on decedents who's religious beliefs forbid it was passed on September 30th by the California State Legislature. The Bill, introduced by State Senators Rosenthal and Robbins provides that an individual may execute a "certificate of religious belief" stating that voluntary autopsy violates the religious convictions of the person. The certificate must state "in clear and unambiguous language that any postmortem anatomical dissection or that specified procedures would violate the religious convictions of the person. The certificate shall be signed and dated

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(6)

by the person in the presence of two witnesses. Each witness shall also sign and date the certificate and shall print on the certificate his or her name and residence address."

The new law does not exclude people from medico-legal autopsies--in other words autopsies where the cause of death cannot be established or where there is a suspicion of homicide or suicide.

We are a little puzzled by this law (as was one attorney we spoke with

about it) since the California Health and Safety Code already provides that directions in a will prohibiting voluntary autopsy are also valid. The intent of SB 1824 appears to be to allow people who have not executed a will and who have religious objections to autopsy to exempt themselves by a simple statement to that effect.

It is not clear how applicable this law will be to cryonicists since it is specifically requires that "The court shall set aside this certificate if it finds that the certificate was not properly executed or that it does not clearly state the decedent's religious objection to the proposed procedure (emphasis ours). For cryonicists, the safest course is still probably to give such directions in a will and cryonics paperwork.

It is interesting to note that this legislation was pushed through by a small and determined group of fundamentalists who mustered both the financial wherewithal and the personal persistence to make it a reality. When will we learn to do the same?

SCIENCE UPDATES by Thomas Donaldson

#### COMPLAINTS ABOUT NEURON GROWTH IN BRAINS ARE WITHOUT FOUNDATION

Recently in CRYONICS we have reported work suggesting that neurons will divide and make other neurons in the brains of higher animals, both mammals and birds. One of the more spectacular experiments of this kind is one by Nottebohm and Goldman (PROC NATL ACAD SCI 80, 2390 (1983)), who demonstrated dividing neurons in the brains of adult canaries.

However, particularly for scientists who believe in a dogma that neurons cannot divide, complaints exist about these proofs. For instance, synapses can occur on the other major type of brain cell, the glial cells. Identification of neurons can be ambiguous. Someone will always come forward to say that if it divides it cannot be a neuron because neurons don't divide.

Fernando Nottebohm and J.A. Paton have now presented some further experiments which quite conclusively show that these new cells (which result from dividing neurons) act physiologically and electrically like neurons...and therefore should BE neurons. (If it quacks like a duck, walks like a duck, and has feathers, it's a duck!)

They gave adult canaries radioactively labeled thymidine, which is incorporated in new DNA during cell division. The presence of labeled thymidine in a cell will therefore show that cell to have resulted from recent cell division. Nottebohm and Paton then recorded the electrical potentials of individual cells in the canary's brain.

Neurons consist of a central nucleus and long "wires" (the axons) connecting them to other cells, and scientists have a way of tracing back along

(continued on page 36)

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(7)

#### BAY AREA UPDATE

by Dick Marsh

This is what's happening:

Money. Things were looking for Trans Time for a while, but then they began to look up -- more or less. In April TT lost \$2097 (\$640 as

depreciation). Revenues from emergency responsibility and long term storage were not spectacularly good, to say the least. Then the Nikolic family of Yugoslavia came to the rescue, demonstrating their love of life and their distaste for death by sending \$5359, of which \$2706 was used to meet expenses incurred in an attempt to arrange for suspension of a family member. They also became full suspension members of BACS. Result: a "positive" Trans Time financial picture for June appeared to be developing.

But the suspension attempt was suspended when the Nikolics were unable to raise the full \$80,000 recommended for the complete process. the remainder of the \$5359 will be returned to the Nikolics.

Earlier, however, John Day brightened the economic picture a bit by repairing the insulating lid on the Andonian capsule so that it is now performing more effectively.

But, says Art Quaife, we need about one suspension a year to break even. Failing that, we fall behind.

Public Issue of Trans Time Stock. First, the good news. Trans Time revealed at the April 29th meeting of its shareholders that it had "sold \$4000 worth of stock to an investor who wished to supply seed money for a public issue of. . . stock. He suggested that Trans Time attempt to raise \$2 million by the issue. A Prospectus and a Business Plan are thus in preparation."

And now the bad news. Ms. Schuyler Williams, an attorney for the prestigious San Francisco law firm of Pillsbury, Madison, and Sutro, has suggested to Art Quaife and Paul Segall that this is a bad time for a public issue of stock. But she further suggested that it may be possible to secure venture capital or private investment, and she mentioned several associates who might be helpful to TT in its quest for funding. The TT board agreed to remain in contact with attorney Williams.

BACS Legal Health. It's basically good, according to a report by attorney Jim Bianchi, who has conducted an extensive investigation into the matter. The report, made in May at the Lake Tahoe meeting, did however mention a few shaky areas. Among them are these: the need for clarification in the By-Laws of the exact responsibilities of each director and officer, the need for a Resolution Book in which such responsibilities would be recorded, and the need for a declaration of minimum standards in dealing with all cryonic suspension.

Jim noted further that Trustees of the Suspension Funds are particularly vulnerable, that record keeping is extremely important, that records should supply information about all aspects of patient care and condition including that found in pre-suspension medical records, that authorization to make changes should be obtained from surviving relatives, and that BACS should maintain the option of changing the suspension services provider if that becomes necessary.

Bianchi felt that the conflict of interest problem inherent in the interlocking BACS-Trans Time directorate has been resolved somewhat by the

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(8)

election of a disinterested majority to the BACS Board of Governors. He recommended that a complete disclosure of anything suggesting conflict of interest should appear in the minutes, a copy of which should be routinely



sent to the Attorney General's office along with an explanatory letter.

In general, however, Jim felt that BACS's legal health was good.

Publicity. Art Quaife sold 20 photos to "Paris Match" magazine for \$500. The magazine wanted illustrations for an article about the now-well-known French gynecologist who had stored his deanimated wife in a deep freezer in his basement. When the freezer went out, as you doubtless know, the repairman blabbed to the authorities, who demonstrated the state of French legal thought by demanding burial for the wife.

Incensed and loyal, the doctor is fighting back. He has said that he will battle the bureaucrats in court and, if he loses, seek to have the body (with its slumbering consciousness) moved to another country, where it can be respectfully suspended.

Anticipating contact by the determined French physician and foreseeing the possibility of similar situations throughout the world, Trans Time has created a committee to develop a flexible funding policy adapted to such emergencies.

Art has also been on the air again. First, in a five-minute phone interview on "The Question Show" by Jim Nayder of the PBS station WBEZ-FM in Chicago. Then, in a 15-minute phone interview on KMBZ radio in Kansas City, Missouri, during which the name and address of Trans Time were worked in twice.

Earlier -- in late May -- KGO-TV in San Francisco broadcast a taped interview with Paul Segall. Still earlier -- in April -- Trans Time maintained a display booth at Marin County's "Future Expo" featuring charts, photographs, and other visual displays.

The July 22 edition of "Sunshine," the Sunday magazine of the Fort Lauderdale "News-Sentinel," carried a story on cryonics and a Trans Time photo.

In August, the author/editor of "Bay Area Update" discussed life extension and cryonics on two audience talk shows: the half-hour "Fighting Back," hosted by June Kessler on KUSF-FM, and a two-hour version of the popular "New Dimensions" hosted by Michael Toms and shared with psychotherapist Mel Krantzler on KALW-FM, a San Francisco PBS station supported by the San Francisco Public School System. The phone light was constantly blinking because of listeners phoning in their questions and comments -- none hostile, most intelligent. The "New Dimensions" series is broadcast via tape cassettes by 50 AM and FM stations across the country, and the cassettes are also sold privately in large number. Both the Trans Time and the BACS phone numbers were worked into the program.

The "Update" author/editor also made a presentation on cryonics to a class in "Death and Dying" in the Oakland High School. This is not a world-shaking event, but it is worth reporting because of its enthusiastic reception. The students, mostly black, listened eagerly, asked extremely penetrating questions and, in a follow-up survey, chose the cryonics presentation as their favorite guest talk of the semester because, unlike the others, it made them feel optimistic instead of depressed and pessimistic.

When the suggestion was made by the speaker that next semester the course title be changed from "Death and Dying" to "Life and Living" the

suggestion was listened to by teacher Judith Yeager with more than tolerant politeness. . . .

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(9)

Maybe there's justice in the universe after all. Consider this report by Paul Segall: "The recent fiasco in Amador County involving the dumping of the ashed remains of approximately 9000 individuals on a vacant tract of land, when they were supposed to have been scattered on Sierra mountain tops from an airplane, has proved an embarrassment to cryonics opponent John Gill, Secretary of the California Cemetery Board. In an interview reported by Jerry White, Mr. Gill appeared to be under pressure for not having done more to avert this disturbing deception." Just what the 'pressure' may be is not clear, but perhaps one can be forgiven for hoping it is proportional to Mr. Gill's opposition to cryonics.

Publicity to come? At the July 29th Trans Time meeting, BACS Vice President Ron Viner suggested some possibilities for holding a cryonics fair. With lightning rapidity, he found himself on a committee, with John Dary and Paul Segall, charged with investigating the potential of such a fair. Earlier, Ron had manned (personed?) a cryonics publicity booth with BACS President Jack Zinn in the San Francisco Airport.

Cryonics Building Fund. Trans Time needs new quarters. The lease on their present quarters is about to expire, and the landlord's renewal terms are unreasonable. Besides, more space is needed -- not to mention freedom in general from the vicissitudes of renting. So, as explained in the last issue of Bay Area Update, a fund has been started for buying a building.

More than \$25,000 has been raised, and a possible site has been discovered. In the words of John Day, "it is a wonderful old mortuary in a very goo commercial area in Oakland. It turns out that a lot of mortuaries are going out of business these days, and a mortuary building is not much good for general usage, so prices are about the same as for bare land. The one we are looking at is larger than we need now, and has a larger parking lot which we do not need, but would be quite suitable for storage, research, or even a storefront type operations of some sort. Because of the large parking lot the price of the whole property is out of reach, but we hope to make a deal with a building to split the property in such a way that we could afford the portion with the building."

Aggressive attempts are being made to find such a builder. Meanwhile, John explains, the Building Fund needs more pledges to give the "leverage" necessary to make the deal work. "It looks like we can borrow money, some of it at very reasonable rates, but we need more risk takers. I believe the benefits are more than adequate to attract enough capital if we can get the story out to the right people. . . . I hope that enough people who may be thinking of making additional deposits in the Cryonics Building Fund will do so now, so that we can have confidence that when we issue a prospectus the deal will go through."

John's address: John R. Day, Custodian, Cryonics Building Fund, 7710 Huntridge Lane, Cupertino, CA 95014. Phone: (408) 255-8460.

Meanwhile, Paul Segall has "mentioned a proposal" whereby Trans Time or the Cryonics Building Fund would receive \$40,000 in equity of a 12% annual, interest-bearing, \$80,000 real estate note secured by a 12,000-square-foot lot in West Berkeley already approved by the Berkeley City Council for 16 units of condominium construction. "This portion of the note," Paul

explains, "would be granted in return for \$20,000 cash, 1/4 interest in a potential building secured for cryonics purposes and 1000 shares of Trans Time stock." The deal is pending.

The Legal Manual: Who Gets What? Trans Time has approved a motion relating to differences with BACS about the legal manual and will wait for their response. Jerry White's summary of that motion, as reported in Trans Time's July 1 minutes,

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(10)

follows:

- "1. Trans Time [will] pay \$100 to BACS for all present and future rights BACS may have to proceeds from Trans Time's distribution and sale of the Manual.
- "2. Trans Time and BACS are both equally entitled to use the Manual as they see fit.
- "3. Trans Time will supply an estimate of upgrading costs and forgive any upgrading expenses.
- "4. Trans Time will supply BACS with a free upgraded copy of the Manual."

Training Session Considered and Postponed. A proposed training session to be directed by Jerry Leaf in Northern California and involving the hypothermic total body washout and attempted subsequent revival of an anaesthetized dog has been deferred until a new Trans Time facility is acquired.

BACS Initiation Fees. It's going to be easier to join BACS in the immediate future -- that is, if a plan proposed by BACS President Jack Zinn and supported in "spirit" by the Trans Time Board is approved by the BACS Board. The new BACS Full Membership initiation dues payment plan would provide the option of paying the \$1000 fee at the rate of \$300 a year for four years. The new plan would be experimental, would be tried for a six-month period, and would not replace any existing plan.

BACS Newsletter. Paul Segall has announced the creation of BACS NOTEBOOK, a newsletter edited and so far largely written by him. It will be mailed with the BACS minutes following each bi-monthly BACS meeting. It will report research and business items of interest to BACS members, and include articles on cryonics and life extension by BACS governors, members, and other contributors.

The BACS NOTEBOOK is intended to have both an educational and a public relations function. The hope is that, as it grows, it will be informative, inspiring, and -- we can hope -- a bit entertaining. Beyond that, it may also give its readership a clarified sense of what BACS is -- in Paul's words -- "all about."

The first issue -- May-June, 1984 -- is loaded with information about research activities in the Bay Area. Only the most superficial summary is possible here. Among the items discussed are these:

1. A May 31st "Progress Report" sent by BioPhysical Research and Development (BPRD), a BACS research contractor, to Saul Kent of the Life

Extension Foundation (LEF) of Hollywood, Florida.

Reason for the report: In January, 1984, LEF awarded a \$5000.00 grant to BPRD "to investigate the total body washout of hypothermic hamsters, and to study the possibility of these animals surviving liquid state conversions."

Highlights of the report: BPRD researchers accomplished the following:

\*\* Developed several techniques for machine-controlled artificial respiration and blood substitution perfusion.

\*\* Improved arterial cannula design and upgraded the technique of venous cannulation.

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(11)

\*\* Maintained co-operation with the Department of Physiology-Anatomy at the University of California.

\*\* Conducted more than 35 individual experiments involving the perfusion of hypothermic hamsters, thus becoming "more familiar with the complicated surgical techniques involved" and discovering "some important correlates of survival following this arduous and unusual procedure."

\*\* Successfully investigated the use of ketamine to replace the closed jar technique in anaesthetizing hamsters. Revived two ketamine-anaesthetized hamsters following total body washout near the ice-point. Established the possibility of using ketamine to prepare for the induction of hypothermia by whole body immersion in crushed ice and thus avoid possible injury due to a deficiency of oxygen in the tissues and an excess of carbon dioxide in the blood.

\*\* Pioneered new techniques of blood collection, using heparin injections into the heart of the donor-hamster to prevent clotting and therefore allow the harvesting of more blood. Developed ways of reducing the number of hamsters required for sacrifice, shortening preparation time, allowing the use of fresh blood during each perfusion, and eliminating the complications of blood storage from many experiments.

Much of this research was presented on videotape at the Lake Tahoe Life Extension Festival in May, and an abstract of the research was presented in August at the Cryo 84 Conference sponsored by the Cryobiology Society in San Diego.

## 2. Highlights of a June 7 "Progress Report" from BPRD to LEF:

Paul Segall and University of California Senior Sandra Gan have:

\*\* Surgically interfaced the circulatory system of hamsters with three-way stopcocks, thus "allowing for transducer measurements of both arterial and venous blood pressure during the induction of and the revival from deep hypothermia. . . effluent at any time during the procedure, thereby permitting determination of pH, chemical constituents, blood gases, hemoglobin concentration, hematocrit, and other information as needed."

\*\* Adapted thermistor leads "on-line to a recording polygraph, providing a continuous record of deep body temperature."

In addition, BPRD researchers have:

\*\* Introduced several new surgical variations (partly in cooperation with Pacific Medical Center biochemist Dr. Hal Sternberg). One of these is a simplified method of warming the chilled animals. This "takes. . . advantage of the simple but ingenious design of Dr. Harold Waitz's operating stage. . . allowing for larger temperature changes without the necessity of moving the hamster being operated on. The importance of this can not be over-stressed, as moving animals while instrumented and intubated microsurgically has rarely been done without extreme risk to the entire experiment." Dr. Waitz is also involved in regenerating a blood gas apparatus borrowed from Trans Time, the use of calcium blockers in prolonging the lives of oxygen-deprived hamsters, and the use of a computerized accounting system.

\*\* Granted permission to a Los Angeles television crew to videotape several of the experiments.

\*\* Agreed to be interviewed for a PBS documentary on life extension.

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(12)

\*\* Begun preparations for an anticipated presentation at the Cryobiology Conference in San Diego at the end of August.

\*\* Accepted a donation from the Foundation For Infinite Survival of several mice of a strain commonly used in aging research. These have grown into a colony of nearly 25 mice, which will be used to test a new theory of aging developed by Paul Segall -- following conversations with Berkeley graduate student Greg Cole -- on the basis of a hint contained in an article by Dr. Roy Walford.

\*\* Begun planning the use of the new mouse colony as a source of animals for a Mouse Cloning Project. BPRD researchers will be joined in this Project by Dr. Susan Weintraub, formerly of Cutter Laboratories, the University of California, the Cornell University Medical Center.

\*\* Begun negotiations with Trans Time for the acquisition of work space in the new facilities being sought by Trans Time.

3. Been informed of the approval for presentation of an abstract of a BACS-Sponsored research project at the Cryobiology Society's Cryo 84 Conference in San Diego on August 21.

The abstract, entitled REVIVING HAMSTERS AFTER HYPOTHERMIC ASANGUINEOUS PERFUSION, summarizes an experiment in which five hamsters were revived following total body washout at temperatures within a few degrees of the ice point. Four of the hamsters were put into hypothermic states by being placed in crushed ice following the closed jar technique and one after injection with ketamine, as discussed earlier in this issue of "Bay Area Update." The animals survived 1-6 hours after regaining consciousness. Hemorrhagic shock due to anti-coagulation and surgical trauma are suspected as the eventual cause of death.

4. The submission of an abstract of a new theory of aging for presentation at the October 18th meeting in New York of the American Aging Association (AGE). This is mentioned above in the discussion of the mice colony.

The abstract, entitled AGING AS A PROGRAMMED CASCADE OF SPECIFIC CELL DEATH, summarizes some of the theorized connections between certain dietary restrictions and delay of aging.

In addition to the research activities briefly summarized above, BACS NOTEBOOK discusses Trans Time's search for investors and reprints a lively new promotional piece designed by BACS "to broaden its base of public support." The piece is entitled WHAT MOST PEOPLE HAVEN'T HEARD ABOUT CRYONICS. It discusses, in nontechnical language, the preservation of endangered species, the protection of patients needing transplants, whole body storage of organ donors, emergency space medicine, the delay of the aging process, and the opening of new frontiers in surgery.

If you attended the Lake Tahoe Life Extension Festival in May, you may have read this piece: it was published in the printed program. If you could have attended the festival but did not, you made a big mistake.

I, too, have recently made a big mistake. I got mixed up about deadlines and so have tried to jam an ungodly amount of stuff into this issue of "Bay Area Update." Sorry. It's all straightened around in my mind now, and next time I'll try to be briefer, and better.

(13)

#### Histological Study of a Temporarily Cryopreserved Human

The case history of the present subject, referred to as P3 elsewhere (1), has been previously described. Essentially, the patient was cryopreserved a significant time after clinical death and was stored for several years in liquid nitrogen until it became necessary to terminate cryopreservation most of the patient's body. After thawing of the non-cephalic portion of the body, tissue samples were taken and were preserved in either Karnovsky's fixative (1) or in buffered formalin fixative (1) and processed some time later for standard light microscopic observation. The results represent the first direct information ever obtained concerning the effects of cryopreservation, as carried out under real working conditions, on the cellular and noncellular structural integrity of the human body. Although the conclusions which can be drawn from this single case are limited in several ways as will be described in the discussion which will follow, the results are such as to provide considerable encouragement for those individuals who now are or who someday will be considering cryopreservation as a personal alternative to death.

#### MATERIALS AND METHODS

Removal of Cryoprotectant from Tissue Samples. All tissues were fixed as described elsewhere (1) on January 4, 1984. The tissues were then stored at room temperature until March 19, 1984. On this date the tissue samples were decanted, blotted, and weighed and the volume of fixative used to store each tissue sample to this point in time was similarly measured. The results are shown in Table 1. In all cases, the volume of fixative used was more than 10 times the weight of the tissue sample. At this time, the liver samples were

Table 1: Sample Preparation for Histological Observation

1ø fixative*	:	K	F	K	F	K	F	K	F	K	F
Tissue type**	:	Kid.	Kid.	LV	LV	Lung	Lung	Liver	Liver	SC	SC
Wt. of tissue****	:	0.57	1.24	0.275	0.50	0.56	0.33	0.38	0.55	0.59	0.42

ml 1ø fixative	:	9.4	14.8	8.1	8.6	8.3	7.5	8.2	13.2	6.9	6.2
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For first dilution (a):

VPFR (ml)	:	0	5	0	0	0	0	0	3	0	0
VDA (ml)	:	9.9	10.8	8.3	9	8.7	7.8	8.5	10.6	7.4	6.5
FVAFD (ml)	:	19.3	20.6	16.4	17.6	17.0	15.3	16.7	20.8	14.3	12.7

For second dilution, one-half of the FVAFD was replaced with fresh diluent.  
For final dilution and details of osmication, see text.

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\* 1ø = primary; K = Karnovsky's fixative; F = formalin fixative.  
 \*\*Kid. = kidney; LV = left ventricle of the heart; SC = spinal cord and spinal nerves. \*\*\*Weight of tissue samples after blotting, given in grams.  
 (a)VPFR = volume of 1ø fixative removed; VDA = volume of diluent added;  
 FVAFD = fixative volume after first dilution.

floating on their respective fixatives and appeared radically abnormal in color (greenish white). The fixatives for these samples were also quite turbid, in contrast to the transparent appearance of the fixatives bathing the remaining samples.

It was assumed that each tissue sample was impregnated with roughly 3 molar glycerol, which should be washed out gradually to avoid any significant

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(14)

possibility of osmotic distortion of the tissue. The first dilution employed was intended to reduce glycerol concentration to one half of the initial value. However, this initial value was not known with certainty, nor was it certain that all tissues contained the same initial concentration of glycerol. Consequently, it was decided to dilute all samples by precisely the same factor rather than diluting them to precisely the same final concentration. Millonig's buffered formaldehyde fixative (MBFF, modified from the original formula; composition given in Table 2) was therefore added to the original fixatives in measured volumes, taking into account both the volume of original

Table 2: Composition of MB, MBFF, and Phosphate Buffer

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A. MB (Millonig's buffer, modified)

Dissolve 1.42 grams of Na<sub>2</sub>HPO<sub>4</sub> (MW 141.96; final concentration, 99.83 mM), 0.33 grams of NaH<sub>2</sub>PO<sub>4</sub>.H<sub>2</sub>O (MW 137.99; final concentration, 24.17 mM), and 0.082 grams of sodium chloride (MW 58.44; final concentration, 14 mM) in distilled water and bring to 100 ml with distilled water. The resulting solution has an osmolality of about 294 and a pH of about 7.35.

B. MBFF

1. Make 100 ml of double-strength MB.
2. "Dissolve" 7.4 grams of paraformaldehyde powder in distilled water, bring to 100 ml with additional distilled water. Heat to 60-70°C. Add 2N NaOH dropwise until solution mostly clears. Cool to room temperature. Carry out step 2 in a hood using mask and gloves to avoid inhalation of powder.
3. Add 100 ml of double-strength MB to the 100 ml of 7.4% formaldehyde. Set pH to 7.4 Add concentrated CaCl<sub>2</sub> dropwise, with stirring, until a precipitate forms. Filter the solution, label it "3.7% MBFF," and refrigerate. Osmolality: about 1640 mOsm.

C. Phosphate buffer

Dissolve 1.65 grams of Na<sub>2</sub>HPO<sub>4</sub> (final concentration, 116.2 mM) and 0.33 grams of NaH<sub>2</sub>PO<sub>4</sub>.H<sub>2</sub>O (final concentration, 24.2 mM) in water, bring to 100

ml. pH = 7.4, osmolality = 304.

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fixative present and the approximate volume of tissue water/glycerol present (assumed to be 80% of tissue weight). In cases for which the test tubes could not accept the required volume of MBFF, a measured volume of original fixative was discarded and the amount of MBFF needed was corrected and added. The exact details are given in Table 1. After the first dilution step, the samples were stored near 0°C and inverted several times on March 22 to ensure thorough mixing. The second dilution step was carried out on March 27. This time the dilution was performed by discarding one-half of the volume of fixative present in each tube and replacing it with MBFF. The third and final dilution step took place on March 30. This dilution was made by decanting the tissues and transferring them to the cryoprotectant-free solutions described in the next section.

Osmication and Further Processing. At the time of complete cryoprotectant washout, all tissues were separated into two additional categories, tissues to be osmicated and tissues to be processed further without osmication. Tissues not designated for osmication were decanted and placed into the 10 ml of MBFF containing no glycerol. The remaining tissues were decanted and placed into 10 ml of MB containing neither glycerol nor fixative. The latter solution was

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(15)

\*\*\* TYPIST'S NOTE: THE FOLLOWING NINE PAGES (15-23) CONTAINED PHOTOGRAPHS OF HISTOLOGICAL PREPARATIONS. \*\*\*

Figure 1. Pathological area of P3's liver, showing numerous cavities reminiscent of ice crystal spaces. Scale bar = 40 microns. H&E (hematoxylin and eosin stain), Karnovsky's.

Figure 2. "Island" of well-preserved cellular structure in the liver. H&E, Karnovsky's primary fixative. Scale bar = 40 microns.

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(16)

Figure 3A. Lung. For discussion, see text. Giemsa, Karnovsky's. Magnification as in Fig. 2 (M=F2).

Figure 3B. Lung, showing normal smooth muscle (arrowheads). Giemsa, Karnovsky's. M=F2.

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(17)

Figure 4. Kidney. For discussion, see text. PAS (periodic acid/shiff stain), Karnovsky's. M=F2.

Figure 5. Kidney. For discussion, see text. PAS, Karnovsky's. M=F2.

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(18)

Figure 6. Renal arteriole. Fundamentally intact structure is apparent. PAS, Karnovsky's used as primary fixative. M=F2.

Figure 7. Kidney. For discussion, see text. PAS, Karnovsky's. M=F2.



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(19)

Figure 8. Renal medulla. For discussion, see text. H&E, formalin. Magnification as in Fig. 1 (M=F1).

Figure 9A. Left ventricle. Myofibrils are intact but separated. H&E, formalin primary fixative. M=F2.

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(20)

Figure 9B. Higher magnification taken from the center of Fig. 9A. Note the evidently normal and plentiful muscle cross-striations. H&E, formalin.

Figure 10A (left) and 10B(right). A shows cross-section of myofibrils, displaying normal shape and density. B shows a cardiac blood vessel with fundamentally preserved structure. H&E, formalin. M=F1 for both A and B.

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(21)

Figure 11. Spinal nerve. M=F2. Inset (higher magnification) shows more detail. Osmium plus H&E (O+H&E), Karnovsky's.

Figure 12. Second spinal nerve, showing both cross-sectional and longitudinal views of the myelinated neurons. Myelin and axons are intact. O+H&E, Karnovsky's. M=F2.

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(22)

Figure 13. Spinal cord. A (left): cavities in center of cord. B (center): osmicated area in center of cord, showing a few surviving axons of small diameter. C (right): two very well-preserved neurons in center of cord. A and C: H&E; B: osmium + H&E. Karnovsky's for A-C. M=F1 for A-C.

Figure 14. Spinal cord: transition zone from central to outer areas. Note apparently intact central blood vessel (V). Osmium + H&E, Karnovsky's. M=F2.

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(23)

Figure 15. Body of the peripheral part of the spinal cord. O + H&E, Karnovsky's. M=F1.

Figure 16. Outer edge of the spinal cord. Note the two apparently intact blood vessels (V). Osmium + H&E, Karnovsky's. M=F2.

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(24)

replaced the next day with 4 ml of fresh MB. The osmolality of the first Millonig's rinse was 334 mOsm as measured at this time (March 31). Tissues designated for osmication were transferred to 9 ml aliquots of 0.5% OsO<sub>4</sub> in isotonic sodium phosphate buffer (formula given in Table 2) on April 2. At this time, the osmolality of the second Millonig's rinse was found to be

309 mOsm. The tissues were allowed to remain in osmium until April 8th, at which time they were rinsed with 2 ml of phosphate buffer and allowed to soak overnight in 7 ml of fresh phosphate buffer. On 4/9/84 the buffer was replaced with 4 ml of MBFF and sent to American Histolabs, Inc. (Rockville, MD) for parafin embedding, sectioning, mounting, and staining.

## RESULTS

All tissues were examined both with and without osmium postfixation. However, in all cases except for that of the spinal nerves and spinal cord, the non-osmicated tissues were deemed to be the most revealing. Consequently, only osmicated tissue sections will be presented here except for the special case of spinal nerves and spinal cord. All tissues were also examined after primary fixation in either Karnovsky's or formalin (giving 20 experimental groups examined in all: 5 tissues times two fixatives times two categories for osmication or non-osmication). However, the histological results with Karnovsky's appeared to be identical to those obtained with formalin. Consequently, no attempt will be made below to systematically present results based on any one of these two primary fixatives. Except as noted below (for heart and spinal nerves), the original magnification was the same for all photomicrographs and was 100X before photographic enlargement.

1. LIVER. As noted above, the gross appearance of P3's liver was extremely abnormal. The histological results bear this out. The liver consisted primarily of cavities are not, however, tell-tale signs of former ice crystals but instead are necrotic areas caused by the premortal pathology of P3. If this interpretation is correct, then these cavities reveal little about the histological consequences of freezing. Much more revealing, in this case, is the presence within the liver of "islands" of apparently well-preserved structure. Such an "island" of preserved cellular structure is shown in Figure 2. The nature of the cellular structures represented within these "oases" is not clear. What does seem clear, however, is that the degree of structural preservation is superb. The impression obtained from these areas is one of ultrastructural level/molecular level preservation, although the several processes of photographic reproduction involved in creating the image displayed in Figure 2 create a less compelling degree of clarity and "crispness" than is apparent to the naked eye during direct observation through the microscope. The degree of preservation observed is all the more impressive considering the insults suffered by P3 in addition to freezing and thawing.

2. LUNG. The histological structure of P3's lung was far less affected by pathology than was the liver, and consequently appears much more normal. Two views of P3's lung are shown in Figure 3. The lung has the typical thin-walled alveolar compartmentation pattern of normal lung (2) (A). Intact red blood cells (circled) restrained normally within apparently intact capillaries can be seen occasionally and in some lung areas (not shown) were abundant. It was also possible to observe apparently normal smooth muscle (Fig. 3B, bracketed by arrowheads), whose characteristically thin nuclei give the typical zebra-stripe look of smooth muscle. Note the crisp, intact appearance of cell nuclei in all areas of the lung (arrows). Much of the alveolar structure

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(25)

appeared flattened, suggesting atelectasis, but this of change would be more likely to result from the patient's known (1) pre-mortal pathology than from freezing and thawing.

3. KIDNEY. The kidney presented a variety of different and striking appearances. Figure 4 shows one type of appearance in which the renal

tubular cells appear to be torn. Note, however, the intact-appearing cell nuclei in these structures (circled). The characteristic peritubular basement membrane (arrows) appears to be intact. The glomerulus (large structure just to the right of center) presents a surprisingly and impressively normal appearance and displays an intact's Bowman's capsule. Figure 5 shows a similar area in which the tubules are shown in longitudinal section. Not only do the cells appear to be literally torn apart, but they are separated from the basement membrane. Note also the presence of an extensive amount of unidentified ground substance (G) filling the normally empty interstitial space. The presence of this material presumably reflects pre- and/or postmortal pathology rather than any change produced by freezing and thawing. Figure 6 shows an apparently intact arteriole surrounded by torn tubules. The second type of renal appearance is shown in Figure 7. Here the tubules do not appear to be torn and thus appear more nearly normal, but their overall appearance and the presence of material in the tubular lumina suggest that they are necrotic. The cell nuclei and the tubular basement membranes, however, appear intact, as does the glomerulus. Again, ground substance fills the interstitial space. Finally, the third appearance of the kidney is shown in Figure 8. This region, from the renal medulla, shows strikingly normal and intact appearing tubules and ducts, although there is an equally striking contraction of most tubules with separation from their surrounding basement membranes.

4. HEART. The appearance of the left ventricle is shown in Figure 9. The muscular bundles shown represent cardiac muscle cells arranged end-to-end and appear to be intact, though they are separated by large extracellular spaces not normally encountered in heart. At the magnification shown in Fig. 9A, and, indeed, at the resolution normally available with the light microscope, the cardiac mitochondria cannot be seen. Muscular cross-striations are also difficult to discern at this magnification, but, as indicated at higher magnification in Fig. 9B (see arrows), they can be seen frequently. These characteristic striations attest to the surprisingly good histological preservation of the tissue and the apparent absence of thaw-rigor. The muscle fibers shown in Figure 9 also appear normal when viewed in transverse section (Figure 10A). Normal blood vessels were also observed in the left ventricle (Figure 10B). In some areas, apparent separation of myoblasts along the intercalated discs was observed, but this was not a constant finding and probably represents tearing artifacts produced by osmication (not shown).

5. SPINAL NERVES. Figure 11 displays the appearance of one of P3's spinal nerves as shown in transverse section close to its point of origin from the spinal cord. The overall structure of the nerve in general and of the myelin sheaths in particular appears strikingly and impressively well-preserved. Unfortunately, many details visible in color are obscured in this black-and-white print and by photographic reproduction. Within the myelin sheaths (dark circular areas) shrunken but apparently intact axons can be seen, with obviously distinct boundaries (shown more clearly in the inset). It should be kept in mind that the observed shrinkage of these axons could represent a fixation artifact rather than an effect of glycerolization or of freezing and thawing. Similarly impressive preservation of another spinal nerve is shown in Figure 12. It is important to point out that these nerves are entirely representative of all such structures observed and were not selected on the basis of unusually good preservation.

6. SPINAL CORD. P3's spinal cord manifested evidence of a severe undiagnosed degenerative condition primarily confined to the center of the

cord. A section through this region is shown in Figure 13. As shown in Figure 13A, the central area of cord appeared to consist primarily of cavities and connective structures. However, in most cases of osmium did not penetrate to the center of the cord, and in the absence of osmium it is very difficult to see myelin. Figure 13B shows a central area of cord in which osmium did penetrate. Here we do in fact see myelin and some small myelinated fibers, but the structure of the cord is clearly degenerated. In Figure 13C, from a non-osmicated region of the center of the cord, two apparently intact, apparently nervous tissue cells can be seen. Their nature is unidentified.

As one proceeds from the center of the spinal cord to the periphery, one encounters a transitional zone between the clearly degenerated regions deep within the cord to strikingly well preserved regions near the cord surface. This transition zone and the outer edge of the cord. This region manifests excellent histological preservation, with intact myelin sheaths and intact myelinated axons, although considerable areas of non-nervous ground substance, presumably related to P3's nervous pathology, are also present. Finally, Figure 16 shows the outer edge of the spinal cord, with typically excellent histological preservation and two apparently normal cord blood vessels which are free of blood cells (indicating that the cord in fact perfused with glycerol).

#### DISCUSSION AND CONCLUSIONS

In considering the meaning of the observations reported here, we will discuss the following three general questions. 1) What can be said to have learned from the observations reported here? 2) Are our observations consistent with, and are they illuminated by, cryobiological findings obtained on simpler systems (particularly whole organs)? And finally, 3) what are the implications of our findings with respect to the repair of freezing damage in cryopreserved humans and the feasibility of cryonics in general?

##### 1. What have we learned from the present investigation?

Caveats. With respect to this question, it is first important to define the limitations of this study. First, the observations were made on a single patient only, and could theoretically be unique to this patient, making our observations no better than "anecdotal." Second, we do not know what the tissue levels of glycerol were in the areas subjected to investigation, so it is difficult to estimate the amount of dehydration and ice formation each of the examined tissues was subjected to and, therefore, the real resistance of the tissues to these stresses. Third, this patient was subjected to devastating premortal pathological conditions directly affecting at least two, and probably all, of the tissues examined, and this pathology, together with the considerable postmortal delay before cryopreservation, not only limited the availability of intact tissue available for examination but also could, in principle, have affected the degree of histological cryopreservation of the remaining intact tissue in either a positive or negative direction. Fourth, this report was not written by a trained histologist, microscopist, or pathologist, so we are not competent to present detailed analyses of the histological results in terms of known pathological effects or even in terms of comparison to normal tissue. Indeed, our present study lacks any control tissues for comparison. Finally, our study does not attempt to look at the ultrastructural

structural or detailed biochemical integrity of the tissues examined and therefore provides information only on a relatively gross level of biological organization.

Results. While fully acknowledging and recognizing the above limitations, major conclusions of considerable importance can nevertheless still be drawn with confidence.

First, current methods of human cryopreservation are capable of preserving a tremendous amount of cellular and non-cellular detail even in patients suffering from extensive pathology and extensive postmortal deterioration and preserved without the desired degree of cryoprotectant permeation into the tissues. Extensive histological detail was observed in every tissue examined.

Secondly, not only the quantity but also the general quality of histological cryopreservation observed is pleasantly surprising and impressive. Apparently intact cell nuclei and blood vessels were present everywhere, seemingly ultrastructural-quality preservation was seen in intact portions of the liver, glomerular and basement membrane preservation and even tubular preservation in selected regions was observed in the kidney, distinctly intact cardiac muscle fibers with apparently normal patterns of striation were found, and the general histological organization of the lung as well as all other tissues examined was intact. Cell membranes and even capillaries appeared to escape gross structural injury as evidenced by distinctly intact red cells containing clearly visible hemoglobin and the fact that these red cells always confined to capillary lumens and were not found in the extracellular spaces surrounding the capillaries. Few clear signs of mechanical distortion and permanent alteration of the tissues by the presence of ice were found, most tissues appearing not to have been frozen at all, despite the fact that extensive freezing did in fact take place. One possible exception to this general rule was the kidney, which exhibited apparent tearing of the tubular cells in a pattern which to the authors' knowledge is not characteristic of any known pathological condition. A perhaps more clear-cut exception was the heart, which contained large and abnormal extracellular spaces which were likely to have resulted from extracellular ice formation. On the other hand, it is also possible that these spaces simply represent cardiac edema induced by cryoprotectant perfusion.

Third, the histology of both central nervous system tissue (spinal cord) and peripheral nervous system tissue (spinal nerve) appeared to be preserved better than the histology of any other tissue. Intact nerve cell membranes and intact myelin sheaths were observed, and there was no evidence of tissue distortion by ice with the possible exception of what appeared to be microscopic fissures in the tissue. However, it is likely that these fissures are artifacts produced by the sectioning of osmicated, parafin-embedded tissue, as osmication always seems to be associated with such features even in tissues which have not been frozen and thawed (data from a separate study). Rather extreme shrinkage of the axons within their myelin sheaths was observed. Nevertheless, the degree of preservation seen was overall, highly impressive and encouraging.

Fourth, and finally, something can be said about the biochemical state of the tissues examined. Histological stains act by chemical reacting with specific types of functional groups in the tissues. To the extent that stained tissue is observed to exhibit both the normal color and the normal intensity of stain expected for control tissue, it can be concluded that it possesses the same functional groups in the same amounts as untreated tissue. We did not find the tissue staining in this study to be discernably different from what would be expected for normal control tissue. Therefore, within very broad limits, we can conclude that extensive chemical modification of human

tissue is not caused by cryopreservation of the body.

## 2. Relationship of current study to the cryobiological literature.

Shrinkage of axons and the spinal cord. As noted above, significant axonal shrinkage was observed histologically. One possibility is that this represents osmotic shrinkage of the axon due to permeation of glycerol into the space between the sheath and the axon without further permeation into the axon itself. Osmotic dehydration might also explain the gross shrinkage of the cord as a whole observed macroscopically (1). Although Menz's data (3) and the data of Fahy et al. (3B) indicate that glycerol permeation into nervous tissue requires only several minutes to an hour at room temperature, permeation is apparently quite slow at 15°C or below (3C). However, if osmotic dehydration by glycerol is the cause of the observed shrinkage, it is remarkable that freezing did not alter the semipermeability of the axonal membrane to permit glycerol entry after thawing. The shrinkage may instead be a fixation artifact, as similar shrinkage has been seen in control animal material fixed without previous perfusion with cryoprotectant. In this case, shrinkage of the cord as a whole could also be fixation-related or it could be attributed to premortal pathology. A more remote possibility could be that freezing caused shrinkage of the axons and that the axons then failed to regain their normal volumes upon thawing due to being injured in some way by the freezing-thawing process. However, there is little or no precedent for this type of behavior in the cryobiological literature.

Mechanical distortion of tissues by ice. In general, little evidence for any mechanical injury to P3's tissues could be found in this study. Although there is currently basic unanimity within the organ cryopreservation field that mechanical injury from ice is a major causative factor in the failure of presently available organ freezing procedures, the observations made here showing little or in some cases no evidence for this type of injury are in agreement with recent studies which point toward ways of avoiding mechanical damage. In particular, considerable recent research associated with the MRC Medical Cryobiology Group in Cambridge in the United Kingdom (4,5), involving both smooth muscle strips and whole rabbit kidneys, has shown that extremely slow cooling, on the order of the cooling rates used for the freezing of P3, causes ice to form in a pattern which prevents or greatly reduces the disruption of extracellular architecture of these systems which is otherwise caused by freezing at normal rates.

Evening at higher rates, however, mechanical injury may not be detectable. For example, a classical study of Meryman's, intended to evaluate the significance of mechanical distortion of tissue by ice, found that even after extreme tissue distortion by ice, as revealed by freeze substitution, thawed liver resumed an essentially normal appearance (6). It has also been reported that the entire leg or foot of various animals can survive freezing to -15°C (or even to dry ice temperatures, according to H.T. Meryman) without the benefit of a cryoprotective agent (7) despite the massive distortion of tissue structure which must be inevitable during such extreme conditions of freezing. It has been known for years that intertidal animals such as snails, oysters, and mussels, which survive extreme conditions of freezing for months at a time, do so despite almost unbelievable mechanical distortion by ice (8), which is not apparent upon thawing. In a review of organ preservation published many years ago, Robertson and Jacob called the histological appearance of tissues after freezing and thawing "unremarkable" (9). Slow freezing of at least 80-90% of the water in isolated canine lungs in the absence of cryoprotection was found to be compatible with survival of the lungs, as determined by acceptable

function after transplantation in many cases and by good histological preservation (10), in agreement with the present results.

In our experience, the appearance of P3's cardiac muscle, which contained exaggerated extracellular spaces presumably produced by the former presence of extracellular ice, is virtually identical to the appearance of frozen-thawed rabbit skeletal muscle as reported by Meryman (7). On the other hand, P3's heart muscle also appears extremely similar to the control hearts of Lillehei et al. (11). Mechanical distortion of the skeletal muscle of human bodies frozen without a cryoprotectant (12) is apparently more severe than what we observed.

The apparent tearing of renal tubular cells reported seems to be without precedent. It seems unlikely to be due to mechanical effects of ice formation. Toldeo-Pereyra, for example, did not observe this type of injury in human kidneys frozen very quickly to nearly the glass transition temperature (13), and tearing similarly appears absent in kidneys frozen very slowly to dry ice temperature (5). It is possible that this injury is a form of microscopic fracturing which takes place only below the glass transition temperature to relieve long-range thermal stresses which cause other organs to fracture macroscopically but which do not in general produce macroscopic fractures of the kidney (1). But this interpretation is at odds with the observations of others (14,15), who did not observe cell tearing even though they froze kidneys to below the glass transition temperature. Perhaps this pattern was somehow produced as a result of premortal pathology, e.g., as a result of the dense ground substance which could have prevented normal thermal contraction of tubules, or perhaps it is an artifact of some kind. Further research will be necessary to elucidate the cause of this unusual type of injury. On the other hand, we found no evidence for histological injury to the renal glomeruli. This type of injury is known to be greatly diminished or prevented at the cooling rates experienced by P3 (5).

We also found no evidence of unravelling and disruption of myelin sheaths as reported by Menz (3) in his study of the freezing of cutaneous nerves, which could be a mechanical effect of freezing. This disagreement is almost certainly because Menz's nerves were frozen abruptly in the absence of cryoprotectant while P3's nervous system was frozen very slowly in the presence of glycerol. Menz's study also showed that dimethyl sulfoxide may cause focal unravelling of myelin similar to that seen in rabbit brains perfused with dimethyl sulfoxide-containing solutions (3C). A very recent study of Jensen et al. (15B) also reported fragility of frozen-thawed rat hippocampal grafts frozen with dimethyl sulfoxide, further suggesting a problem with this cryoprotectant for brain. However, Menz found no such effect of glycerol, in agreement with P3's histological picture.

It seems clear that the findings in this study are in reasonable general agreement with, and are compatible with, other findings in cryobiology concerning mechanical effects of ice (and the lack thereof) on tissue histology.

Thaw rigor. One possible point of disagreement, however, was our failure to observe "thaw rigor," which is rigor mortis produced by freezing and thawing (16). The absence of thaw rigor was inferred from the seemingly normal striation pattern observed (1). It is not clear why we failed to observe the histological pattern of thaw rigor. One possibility is that, in view of the long postmortem delay preceding cryopreservation, the cardiac muscle passed through both rigor mortis and secondary relaxation prior to cryopreservation. Love (16) has noted that very slow

freezing prevents thaw rigor by allowing for depletion of ATP during cooling, which is therefore not available to cause rigor upon thawing.

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(30)

Chemical effects of freezing. Normal histological staining and normal histochemical reactions after the freezing and thawing of kidneys have been reported by others (14). It is also clear that chemical changes produced by freezing must in general be rather limited, or it would not be possible for most cells to survive freezing or for analyses to be made of a nearly infinite variety of biochemical constituents of cells after freezing and thawing, as is commonly done. Our results simply confirm for cryopreserved human bodies, in a limited way, what is already known to be true for the great majority of other systems.

### 3. Implications

The feasibility of reversal of cryopreservation injury. The present results do have an important bearing on the question of repair. The following conclusions seem clear.

First, despite examples in which the observed degree of injury is severe, particularly in the case of the kidney, wherein there appeared to be a physical disruption of the tubular cells, there is never any doubt as to the identity of the tissue being examined. Kidney is obviously kidney, lung is obviously lung, and so on. (Due to the patient's pathology, we are unable to comment definitively on the liver, but it is likely also to follow the same pattern, particularly in view of Meryman's results cited above.) Furthermore, the normal biochemical nature of these easily identifiable tissues appears to be largely unaltered. It follows from these facts that cellular (17) or molecular (18,19) repair machines, if they can be made at all, will have no trouble identifying their environment and proceeding to make appropriate repairs. The observations suggest that the amount of molecular repair required should not be large compared to the overall molecular inventory of the tissue, and therefore that the degree of molecular repair required should fall comfortably within the range of repair capability thought to be possible (18,19). On the other hand, it must be acknowledged that it is not easy to visualize how molecular machines would be able to repair large-scale structural flaws such as those seen in the kidney (torn cells) or those seen on a more gross level as macroscopic fractures (1).

Second, regardless of how much injury was present in a given tissue, even in the case of the liver (in which almost no discernable cell structure at all was present other than what was found in the small "islands"), it was always found that cell nuclei were intact and easy to identify. It follows that the genetic information necessary to identify a given cell and therefore to repair a given tissue will probably be available in practically every cell in the body, despite prolonged periods of postmortal deterioration. This conclusion is supported by the apparent stability of the genome in the face of either freezing (20) or postmortem deterioration (21). Even if DNA is significantly degraded within 30-60 min. of death (22,23), the resulting fragments should still provide ample information for cellular or molecular repair devices to decipher and act upon particularly as these fragments will all presumably be localized within the nucleus.

Third, there was no evidence of catastrophic vascular injury in any of the sections examined. Presumably, then, if macroscopic fracturing (1) can be prevented, the vascular system should in principle be available as a delivery route for both cellular and molecular repair device.

Finally, the tissue of greatest importance, central nervous system and



peripheral nervous system tissue, appears excellently preserved even under the conditions experienced by P3. This observation together with recent studies of the cryopreservation of brains suggests that, in some ways, repair of the

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(31)

brain may be even simpler than repair of the remainder of the body. Naturally, however, much more information on the status of the brain and of the body after cryopreservation is still urgently required. Electron microscopic results obtained on a dog frozen using dimethyl sulfoxide, reported by Gale (24), indicate more CNS damage than is hinted at by the present results with P3.

Overall, this initial study shows that it is feasible to preserve histological detail in humans by cryopreservation after death. Since damage is likely to be more structural than chemical and since this study shows significant structural preservation can be achieved, the results are consistent with general feasibility of Ettinger's proposal for the rescue of contemporary people suffering from incurable terminal diseases (25). Of course, only many, many years of decades of additional research will be sufficient to establish or rule out the true feasibility of this approach.

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#### LETTERS

##### SOME COMMENTS ON RULES AND PREPAYMENT by Thomas Donaldson

When I was very young, only 6 years old or so, and my parents treated me with some form of discipline which I felt at the time was very unfair, I resolved that when I was a grown-up and had children of my own I certainly would never treat them that way.

It is now many years later and for some reason I have completely forgotten exactly what it is that I had resolved never to do.

Recently Mike announced that ALCOR had passed rules on dealing with prepayment. I have some comments on that problem; I DO firmly believe, just as Mike says I do, that prepaid suspension fees should be invested very conservatively. On the other hand, I feel some human sympathy for what is happening.

It is one thing to pass a Rule, and quite another thing to actually observe it when tempted. Many convenient rationalizations exist or can be devised to explain why the Rule really should not apply in this special case, etc, etc. Perhaps even, the Rule can simply be FORGOTTEN when the time comes, a very convenient way of dealing with an inconvenient problem. Or else, the Board of Governors, having passed a Rule, can simply rescind it when a sufficiently tempting case arises.

Furthermore, given the situation of cryonics, there is NO firm rule

which we can produce. If, for instance, it really did become necessary to transfer

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(33)

people to Australia and set up there far from any cities, then we'd have to think carefully about what to do with prepaid funds. That is, not only can rationalizations exist, but if things got bad enough there could be some damn good reasons for spending prepaid funds on capital equipment.

Or in other words, the line between a damn good reason and a rationalization can change depending on who you are.

My own feeling about what to do about such a problem is this. Don't pass any Rules simply because Rules are made to be broken. Just make a habit of PUBLIC deliberations for the committee or other group which handles suspension funds. That is, if you pass a Rule, it shouldn't say how funds should be handled but only say that all discussions on this question should be publicly made, with minutes available for any member of the organization to consult (and perhaps even better, minutes ROUTINELY SENT to every member).

In this way, if there is ever any severe disagreement among the members about the investment of funds, the news will get out and the Trustees in question will be deposed. Otherwise not. If the membership is quite unanimous that prepaid funds should go to (say) a storage house, then perhaps the situation is really so serious that they SHOULD, whether or not that is a conservative investment policy.

In essence, there is no protection in Rules nor in Law. Our only real protection is that the body of members cares for us enough to see that we don't get wasted.

#### PREPAID SUSPENSIONS AND ETHICS by Frank Rothacker

Over the last couple of years, some individuals have expressed concern about the potential problems of prepaid suspensions. It is as though prepayment has problems that are not present in other forms of funding. I disagree with this view. I feel that prepayment poses only a subset of the problems that will eventually be raised when suspensions are paid for by life insurance benefits.

For example, consider the question of how prepaid monies should be managed. Does this question raise any problems that are not also present in the question of how long-term storage monies should be managed? Should prepaid monies be

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(34)

managed any more conservatively than long-term storage monies? If anything, long-term storage fees should be managed even more conservatively, because the suspendee can no longer object to how the money is being used.

What about the cost of a prepaid suspension? Does it stay constant? Those questions have also been debated for long-term storage charges. ALCOR tends to guarantee that a person will stay frozen for a fixed price. Trans Time tends to avoid such a guarantee. What will happen to the frozen subject is a matter of speculation. But prepayment does not add any new problems.

Problems always seem easier if you can postpone working on them until

later. The same is true for the problem of how to manage suspension funds. It is easy to sign up a young member with a life insurance policy and assume that it will be simple to manage the funds when the time comes.

Cryonics organizations have never had to worry very much about how to manage suspension funds. So far, only one suspension member has ever died and left funds to a cryonics group (not ALCOR). Up till then, that particular organization had no plans in place for managing such funds. The treasurer was not even able to deposit the insurance check into the organization's bank account without waiting for the next board of director's meeting.

The one thing about prepayments is that it forces cryonics organizations to deal with problems that they would rather put off until later. But such problems are always part of managing someone else's money. Procedures and ethical standards will have to be developed sooner or later. Because actual suspensions rarely occur, prepayments are one of the few ways that an organization can get experience managing suspension funds before the need actually arises. How well an organization handles prepaid funds is a very good indication of how the organization will handle postmortem funds from insurance. If an organization handles your money poorly while you are alive, you can be fairly sure it will be worse after you are dead.

I agree with those who advocate industry-wide ethical standards. But I also feel that a mechanism for enforcement of such standards will be needed. I have search in vain through the ALCOR suspension documents for an explanation of exactly what they ALCOR do with a suspendee's money. But as far as I can tell, the money is an outright donation to ALCOR and does not legally obligate ALCOR in any way. This is probably being done to maximize the flexibility to the ALCOR board and minimize the possibility of outside interference. But giving such flexibility to the present board of directors also gives similar flexibility to future Boards of Directors. It is uncertain what a future Board might do if no controls are imposed.

ALCOR's suspension forms contain numerous disclaimers. Disclaimers are necessary to inform the public about the limitations of cryonic treatment. I am glad that ALCOR makes these disclaimers, but I am also concerned that some future board might use the disclaimer as a license to do whatever it pleases.

Unlike ALCOR, several other cryonics groups around the country have organized themselves into two separate corporations so that one corporation acts as a trustee for suspension funds and the other corporation provides suspension services. This organizational approach was intended to provide a system of "checks and balances" so that suspension activities and finances would be

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(35)

carefully monitored. But in actual practice, there has always been an intimate relationship between the two corporations that a particular cryonics organization set up. The management of the two corporations usually overlaps and the trustees of the suspension funds are not strongly independent. For instance, there has never been an example of a trustee corporation in one group purchasing services from a service provider of another group.

A few years ago, a cryonics group hired an attorney to review the legal aspects of their suspension procedures and to make recommendations. The attorney produced an extensive set of forms, documents, and procedures to be followed. The new system provided a number of safeguards. But so far, that particular cryonics group has not implemented their attorney's recommendations. In fact, when an elderly person recently signed up with the group, they accepted prepayment rather than use the attorney's recommended trust agreement.

Co-mingling of funds is another ethical problem. There is at least one cryonics corporation that requires customers to pay in advance for some types of services and to post deposits with the corporation. This company co-mingles prepayments and deposits with the corporation's general fund. Although most prepaid services will be delivered within the following twelve-month period, ethical problems always arise when funds are co-mingled. If the company were to go out of business, the customers might not get the services for which they paid.

Financial reports are not readily available. Trans Time keeps regular financial records, but they are available only to directors. BACS occasionally publishes financial reports, but only sporadically. For the past couple of years, I have been asking ALCOR for a financial statement, but so far have not received anything. None of the cryonics organizations have ever had their books audited.

Adding to the ethics problem is the fact that cryonics organizations, in general, do not have well-established procedures for determining policy. Usually, there is nothing in writing which specifies the precise duties of officers and employees. Formal procedures are not used to delegate responsibility or to limit authority. The rules can be changed without a period of public notice and comment.

Mike Darwin is right when he says that the state will eventually get around to regulating cryonics. You can be sure that the regulations will be at least as stringent as regulations for the funeral industry. Funeral directors cannot accept payment in advance. If a family attempts to pay in advance the check will be returned. There can be no package deals. Each item must be sold separately. The customer can refuse anything as long as it is not required by law.

Pre-need arrangements for conventional funerals can be made as long as certain procedures are followed. Usually the customer selects the type of funeral that he or she would like, and after filling out some forms, gives a check to the funeral director. The funeral director deposits the check in a special bank account for pre-need customers. The funeral director manages this bank account and credits the customers with the interest. A management fee is charged. Usually it is about 2% of the interest earned by the account--an extremely low management fee.

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(36)

After the funeral occurs, the funeral director pays himself or herself out of the special bank account. If anything is left over, it goes to the estate. Usually the accrued interest pays for any increases in funeral fees that occurred over the years since the pre-need plan was set up. If interest has not kept up with inflation and there is not enough money, the funeral director selects a less expensive funeral. The funeral director must be bonded to accept pre-need cases, and regular audits are required.

Co-mingling of pre-need funds with the funeral director's funds is prohibited.

Funeral regulations came about because of abuses in the industry. Funeral directors sold pre-need plans, co-mingled the money, and then went out of business before they performed the service. Cemeteries collected "perpetual care" fees as new customers were buried. For years, it seemed to work well while the cemetery kept the lawns trimmed and the flowers watered. But sooner or later, the cemetery filled up and fell into disrepair when no more money came in. Cemeteries became public eyesores and citizens complained to the legislature. Eventually, the state set up the cemetery board to oversee cemeteries and make sure that perpetual care funds were properly managed.

If rotting bodies are ever again found at a cryonics facility, the public will clamor for legislative action. If few other bodies remain in storage at the time, the practice of cryonic suspension will be banned outright. Otherwise, the legislature will give jurisdiction to the cemetery board. The leaders of cryonics groups must recognize this problem and deal with it. They must set ethical standards and provide for enforcement.

(SCIENCE UPDATES continued from page 6)

these axons to the nucleus. They do this by injecting an enzyme, horseradish peroxidase, which they can detect by microscope using the proper dye.

If a cell has an axon which connects to other nerve cells, and if it transmits electrical impulses, then it should be a nerve cell. Nottebohm and Paton showed that some of the electrically active nerve cells which they had traced by horseradish peroxidase ALSO had labeled thymidine in their nuclei. That is, they were the results of recent cell division. Point proved.

This work is interesting and important for cryonics because it tells us something about repair processes. If cells carry out some kinds of repair right now, it follows that such repair is, first, POSSIBLE, and second, does not involve a very great extension over present cellular capabilities. In this particular case, these experiments tell us that duplication of neurons, followed by correct wiring of the brain, doesn't violate any basic physical constraints. It follows that even massive duplication and repair of wiring also doesn't any such constraints: we are not talking about many, many orders of increase in difficulty, but only about a relatively minor (one or at most two) increase in the problem.

Of course, no one can prove possibility except by actually doing something. What these experiments do is to SUGGEST possibility, which is nonetheless highly significant.

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(37)

#### NOVEMBER-JANUARY MEETING CALENDAR

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

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The NOVEMBER meeting will be at the home of:

(SUN, 4 NOV 1984)            Jerry and Kathy Leaf  
                             13152 S. Blodgett  
                             Downey, CA 90242  
                             Tel: (213) 531-2708

DIRECTIONS: From the Long Beach Freeway (Hwy 7), get off on Imperial Highway and go east to Lakewood Blvd.  
From the San Gabriel Freeway (605), get off on Imperial Highway and go west to Lakewood Blvd.  
Go south on Lakewood to Gardendale (1st light) and turn west (right) on Gardendale. Blodgett is the 2nd street on the left.  
Turn left on Blodgett. 13152 is on the left (east) side of the street about midway down the block.

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The Annual Turkey Roast is being co-ordinated by Maureen Genteman. Her home telephone number is: (213) 392-2137  
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The DECEMBER meeting (ALCOR Annual Turkey Roast) will be at the home of:

(SUN, 2 DEC 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 left (north) side of the street.

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The JANUARY meeting will be at the home of:

(SUN, 6 JAN 1985)            Brenda Peters  
                             8150 Rhea  
                             Reseda, CA  
                             Tel: (818) 349-7424

DIRECTIONS: Take Interstate 405 (San Diego Freeway) north into the San Fernando Valley, to Roscoe Blvd. Go left (west) on Roscoe 3-4 miles. Rhea is 2 blocks past Reseda Blvd. Turn left on Rhea. 8150 is the second house in the second block, on the left.