CRYONICS

MARCH 1984 ISSUE # 44

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EDITORIAL MATTERS

We have received several questions from readers for our regular question column, but could use more. We would like to have several months lead time so that we can shop around for the "best" expert to answer any particular question. So, if you have a question you've been thinking about--now's the time to ask! We want to emphasize that questions can be of any kind: technical, organizational, social or legal. We'll do our best to come up with an answer!

The article "Twenty Years of Cryonics" which appeared in the February issue was written by Mike Darwin. A last minute addition to the magazine resulted in a paste-up error obliterating the identifying initials. Those of you who wondered who the "I" was who was mentioned frequently in the article: it was I--M.D.

We urgently need good, nontechnical contributions to the magazine. We are especially looking for thoughtful pieces on the philosophy and history of cryonics. During this year, the 20th Anniversary of the cryonics movement, we would like to see commentary from some of the front-line veterans of "the early days" on what it was like and on where they think we are and/or should be going.

ALCOR FELLOWSHIP

As has often been pointed out, if cryonics is to succeed we at least have to break even. We may not have to grow wildly, but we do have to replace ourselves with capable and qualified people from the next generation. Since the average age of cryonicists is now about 40 and steadily rising, this is a problem which merits considerable attention.

Most of the current leadership in cryonics was recruited young--during grade school, high school or college years. Several of us became as involved with cryonics as we did because of an opportunity to MEET with other cryonicists and to TRAVEL to cryonics facilities and spend some time WORKING on cryonics, or at least getting an intimate feel for how it was being done. For some of us, this meant travelling great distances at considerable personal expense. Obviously, for some really interested, bright young people out there, the opportunity to live and work with other cryonicists for a short period of time is simply not going to be there.

This is a more serious problem than it may at first seem. In the past, both Curtis Henderson and Saul Kent made it a point to travel across the United States with some frequency and to meet with interested people (including high school students) in order to give them a better idea of what cryonics was all about. Owing to circumstances and the changed nature of cryonics operations (with heavier administrative loads and more responsibility) such globe trotting is simply not feasible at this time. What is needed is a way for young people who show real promise to come to Southern California (and hopefully visit Northern California too!) and spend a few weeks or a month immersed in cryonics.

At this time ALCOR is in communication with five young men and women who have shown a sustained interest in cryonics/cryobiology. Several of these

students have shown themselves (through quantity and quality of correspondence and other communications) to be worthy of an effort on our part to recruit them as working cryonicists. For this reason, several of the directors of ALCOR as well as a few other interested individuals have proposed the creation of an ALCOR Fellowship and provided some preliminary contributions towards its realization.

What the Fellowship would consist of is paid, round-trip airfare to Los Angeles, and residence for a period of two weeks to a month (depending upon interest and circumstances) with a local ALCOR member. At this time, appropriate housing and some of the air fare is available. We would like to solicit additional contributions for air fare and if possible, more alternates for housing, should the need arise.

In any event, we hope to be able to offer our first Fellowship by Summer of this year at earliest or by Christmas break at latest. Anyone interested in making a contribution should send donations to: ALCOR STUDENT FELLOWSHIP, 4030 North Palm #304, Fullerton, California 92635. Once we have accumulated enough money for airfare (approximately $400.00) we will provide applications for the Fellowship and detail our requirements.

ALCOR DUAL PATIENT DEWAR REPAIRED

As some of our readers may recall, ALCOR's dual patient cryogenic storage dewar failed to meet the hoped for performance of 10-12 liters of liquid nitrogen boiled off per day. Subsequent to the removal and conversion to neuropreservation of the two whole-body patients who occupied it, this dewar was returned to the manufacturer under warranty (sigh!). The decision to return the dewar was made when it was rolled out onto the tarmac in back of Cryovita during a light, misting rain, and a "cold spot" approximately 18" by 14" was disclosed by condensation of water vapor. Subsequent testing revealed this "cold-spot" to be approximately 10 degrees centigrade cooler than the rest of the dewar's outer skin.

Apparently the inner cylinder of the dewar had shifted off its anchoring trunion during transport and compressed the superinsulation on one side. The manufacturers were able to repair the dewar noninvasively and further hardended the vacuum as well. ALCOR received the dewar back from the manufacturer on the 30th of January and static testing was completed on the 17th of February. We are pleased to report that the repair efforts cut the boil-off rate by half and that the dewar is now performing at a boil-off rate of 8.0 liters of liquid nitrogen per day!

COENZYME Q-10 NOW AVAILABLE IN HEALTH FOOD STORES

Those of our readers who also subscribe to Anti-Aging News or who were present at the 1983 Lake Tahoe Life Extension Festival will already have some familiarity with Coenzyme Q-10 as a potential life extending drug. Both in Anti-Aging News and at the Lake Tahoe Festival Dr. Gregory M. Fahy presented his own research and a summary of the work of other investigators on the life extending effects of CoQ-10, otherwise known as ubiquinone.

CoQ is a critical component of mitochondrial membranes (mitochondria are the "powerhouses" of the cell) and is essential in electron transport in aerobic metabolism. Very low levels of CoQ are associated with heart disease and a

significant decline in CoQ levels has been noted with normal aging as well. In Japan, CoQ is used extensively as a medication for the treatment of chronic heart failure (CHF) because of its effectiveness in improving cardiac output, reducing arrythmias, and improving pumping efficiency of the heart. Of most interest to immortalists is the work of both Fahy and Bliznakov demonstrating up to a 56% extension of the mean lifespan of female mice trated with this agent (Fahy, Anti-Aging News vol. 3 (7): 73-78 July, 1983.)

One of the advantages of CoQ over many other "anti-aging" drugs currently being advocated is that it has an extensive amount of clinical and toxicological information accumulated about it as a result of over a decade of Japanese experience. This is not to imply that CoQ is an innocuous naturally occuring nutrient. CoQ, like most other "anti-aging" drugs (such as the antioxidants in general, and in particular BHT) is also a potent immunomodulator and has been shown by several investigators to act as a restorative to immune systems damaged by chemotherapy or aging (see: Biomedical and Clinical Aspects of Coenzyme Q, Volume 1, K. Folkers and Y. Yamamura Editors, Elsevier Biomedical Press, 1981). CoQ is a powerful drug requiring considerable caution in determining the right dosage for it can depress as well as enhance immune function depending on the dosage used. At this time it is currently undergoing clinical trials by Sandoz for possible release as a cardiac medication in the United States and is also being evaluated at UCLA as a possible treatment for cardiac ischemia in heart attack.

It thus comes as something of a surprise that CoQ has turned up on the shelves of health food stores in Southern California (and presumably elsewhere as well) under the guise of a nutrient. The product, called simply CoQ-10 is being marketed by Twin Lab, a major manufacturer of vitamins and other "life extension" related products. Twin Lab has been very aggressive and amazingly daring during the last year or so and has marketed a number of drugs such as 2-dimethylaminoethanol (Deanol or DMAE) and BHT as nutrients or adjuncts for "home food preservation." The BHT bottles give instructions to add the capsules to cooking oil, but also go to lengths to point out that the capsule containing the BHT is pure gelatin and presumably edible. Of course it also should be noted that one bottle of one hundred, 250 mg. BHT capsules would suffice to treat enough cooking oil to last a family of deep-fry fanatics for years.

So, one way or another, CoQ has become available to people wishing to take it for aging. We might also add that from our understanding of the cost of the raw material from the Japanese suppliers the Twin Lab price of $9.58 for a bottle of fifty, 10 mg. tablets seems very reasonable. The usual dose for heart disease employed by the Japanese is 60 to 120 mg. in two or three divided doses each day. Maintenence doses for use in aging have been put by some "experts" at between 20 mg. and 50 mg. per day.

It is, of course impossible to know if CoQ will significantly extend the lifespan of humans. Certainly, CoQ is a powerful drug whose unsupervised use should be approached with caution. Perhaps the most important thing to realize with respect to the appearance of this agent on health food store shelves is that it stands as a testimonial to the growing power of the Life Extension Foundation in Hollywood, Florida to shape public demand (or at least manufacturer's perception of public demand) for "anti-aging" medications. For those of us with a strong interest in personal use of CoQ, its early appearance in the American marketplace is a pleasant if somewhat mind boggling surprise!

PEARSON & SHAW AND THE MEDIA: IS THE HONEYMOON OVER?

by Simon Carter

"Healthbeat" a nationally syndicated television health news show, carried locally in Los Angeles by KTTV (Channel 11) recently aired a 30 minute long attack on Pearson and Shaw's Life Extension: A Practical, Scientific Approach. The segment which was aired on February 2nd was produced by Metromedia Productions and anchored by a physician, Timothy Johnson, M.D. The hard driving, extremely critical tone of this program would seem to indicate that perhaps the media has now tired of Pearson and Shaw and is ready to provide them and their cohorts with an onslaught of the "other kind of exposure".

The program began by critically evaluating some of the 34 substances the Pearsons' claim to be ingesting. Severe doubts about the safety and efficacy of many of these agents were voiced and a number of experts were trotted out to state that their work had been quoted selectively or even misquoted in the Pearson's book. A spokesman from the Food and Drug Administration hinted at "investigations" and the commentary turned toward the possible fraudulent nature of the book.

With the stage set in this fashion, the picture cut to South Florida where Life Extension Products was introduced as "cashing in" on the Pearson and Shaw inspired "boom" in anti-aging remedies. Saul Kent briefly appeared to quietly defend his products, but could in no way counter the overwhelmingly negative tone of the program.

In a rare appearance on a news show (where no money is offered in exchange for an interview) the Pearsons spoke out in defense of their book and practices. They presented a freaky, almost satanic appearance as they speculated that the basis for the criticism they were receiving was "jealousy" engendered by the financial success which had come their way. Despite our sympathy for the "free market" and a "live and let live" attitude about such matters, it was difficult to feel anything but disdain for the Pearsons as they gloated over their "financial success" and contemptuously disposed of serious criticisms of their work.

The segment ended weakly with Dr. Johnson advising that people would be better off concentrating on good nutrition and abstinence from smoking in order to reach their ordained three score and ten years.

While we at ALCOR have severe doubts as to the effectiveness or even the safety of many of the Pearson's "recommendations" there is a fundamental problem at issue here: can we afford to wait for FDA approval of life extension drugs when such approval will NOT be forthcoming? As cryonicists we are all well aware that sometimes, if we want results we have to take matters into our own hands and take some risks. On the other hand it goes without saying that good judgement and EXTREME caution should be used before any decision is made to take ANY life extension drug. Certainly, it would be hard to imagine a situation where taking 34 of them would be justified.

Unfortunately, if the "Healthbeat" segment does presage an onslaught of media criticism we may all find our options to use anti-aging drugs considerable telescoped. No doubt the FDA will lose little time acting on hysteria generated by the media and will use it as a pretext for sweeping, Draconian action. It's just too bad that "THE BIG BOOK" in popular Life Extension couldn't have been authored by someone with more balance, more common sense and at least a working understanding of the complexity of the problem presented by aging.

ROCALTROL
calcitriol/Roche

A REALLY EFFECTIVE TREATMENT FOR OSTEOPOROSIS?

One of the most crippling and devastating effects of the aging process is the progressive loss of calcium from the bones. This effect is particularly severe in females and is responsible for the "hunched back" appearance exhibited by many elderly women: a consequence of compressive breakdown of the vertebrae secondary to severe calcium loss. Osteoporosis is the 12th leading cause of death in the United States and afflicts approximately 15 million Americans. It is a contributing factor in the deaths of literally millions of others and is a major source of severe and painful disabilities.

Over the years a number of therapeutic approaches have been tried, including the administration of estrogen, since its absence in post menopausal women is known to be a major contributing factor to the illness. Most recently, J. Lane, et al of The Hospital For Special Surgery in New York have reported some success in prevention of osteoporosis with the administration of calcium, vitamin D and sodium fluoride. These investigators found that bone mass and rate of bone accretion could be significantly increased in post-menopausal women with osteoporosis and that fractures could virtually be eliminated after 18 months of treatment.

More recently, several investigators from around the U.S. have reported on a study which indicated that another simpler, and perhaps safer and more effective approach (fluorides are toxic and must be used cautiously in cases of diminished renal function) may work. John Gallagher of Creigton University in Nebraska, Lawrence Riggs of the Mayo Clinic, and Hector DeLuca of the University of Wisconsin recently completed a double-blind study involving 120 people with idiopathic osteoporosis which demonstrated that calcitrol, the active form of vitamin D (which is produced by the kidney) caused a 50% to 75% reduction in fractures. The effects of calcitrol on inhibiting demineralization of bone and initiating remineralization of affected bone were so profound that the control group was switched over to the medication after the first year of the study.

Calcitrol is a relatively new drug first marketed about five years ago by Roche pharmaceuticals. It is an interesting example of how one program of treatment can mushroom into widespread benefits for others afflicted with seemingly unrelated diseases. Calcitrol or Rocaltrol (the Roche brand name) was developed not to treat post menopausal women, but rather to treat a seeminly intractable problem encountered with patients undergoing artificial kidney treatments (dialysis). Because their kidneys have been destroyed by disease, dialysis patients are unable to convert vitamin D into its active form and thus suffer from severe bone demineralization and a pattern of fractures similar to that seen in post-menopausal osteoporosis. Rocaltrol was developed to overcome this deficiency and, since its introduction has greatly reduced the number of bone related complications observed in dialysis patients.

It should perhaps be noted that the 1.2 billion dollars a year being spent on keeping 50,000 dialysis patients alive may just be "cost-effective" (a word Washington health planners love to use) after all if calcitrol proves to be the long awaited effective treatment for osteoporosis.

LETTERS TO THE EDITORS

Dear Editors,

In late December of last year, I received a letter from Anna Tyeb of ALCOR soliciting funds for a mobile cephalarium vault. In addition to the obvious safety aspects of having such a device, it was also stated that it would be useful security-wise if "potential detrimental changes in the legal status of cryonics" were to occur.

While it isn't hard to have colorful and vivid premonitions of desperate car and trailer chases involving those evil cryonics "villians" and the hot-in-pursuit "heroes" of the California Highway Patrol, I think a more reasonable approach would be for interested cryonicists and associates in California to form a political action committee (PAC) to formulate and support appropriate legislation in Sacramento favorable to both short and long-term aspects of cryonics.

Of course, I dearly appreciate the hard work and effort going into the technical development of cryonics facilities. However, it could all be undone by a few strokes of the legislative pen if we are caught with our pants down. I would encourage anyone interested in the PAC idea to contact me at: P.O. Box 1648, Santa Cruz, California 95061 or phone: (408) 425-7151.

I would also like to invite interested cryonics groups and individuals to attend and participate in the Third Annual Conference on Space Development sponsored by the L-5 Society which will be held on the weekend of April 20-22 at the Sheraton Palace Hotel in San Francisco. A golden opportunity to meet other forward-looking people interested in doing something about the future awaits you. Further information can be obtained by writing to:

Third Annual Conference on Space Development
1275 4th Street #242
Santa Rosa, California 95404

Respectfully,
John Krug, BACS Governor
Santa Cruz, California

To Fred and Linda Chamberlain and to anyone else offended by the biography of "Pall Segall" in CRYONICS #42 (January, 1984):

I had not seen the piece in question until I received my own copy of CRYONICS for the month. When I first read the article I thought it was funny in some ways; but I was surprised that Michael had printed something that the real Paul Segall might have interpreted as an unpleasant joke at his expense. When I called Michael and found out that Paul had seen the article and found it funny I went back and re-read it. This time I thought it was an hilarious satiric piece on the quality of much academic writing (If you can't find a subject to write a paper on, write it on nothing. Most people won't know the difference); on Elizabeth Kubler-Ross; and on the strange ways many people become involved in cryonics. Apparently the difference was that knowing Paul said it was okay gave me permission to appreciate the satire.

Satire is the most delicate of the written arts. One step in the wrong direction is bitter; one step wrong in the opposite direction is vacuous. And specialized kmowledge is often required to fully appreciate the humor. The humor of Jonathan Swift's Gulliver's Travels is much more funny to someone who understands the political situation of England in the erly 1700's. The people

who had reviewed the "Seagull" article before publication had the specialized knowledge. They had been given their "permission to laugh" and had been unable to see from the perspective of our readers. As Michael said in his apology last issue, much of the hunor in the article was part of an inside joke, and perhaps inside jokes should stay inside jokes.

Our intention has been to enliven the magazine with lighter pieces from time to time. If one has gone astray, please be assured it was not our intention to hurt anyone's feelings, least of all Paul's.

Sincerely, Steve Bridge
Co-Editor, Indianapolis

Dear Mike Darwin,

Since CRYONICS is published by ALCOR, it has an understandable editorial bent toward "ALCOR first and always". But I wish to take issue with your statement in the February 1984 issue of CRYONICS, page 6: "Because we (ALCOR) have volunteer labor available, and own rather than lease our patient dewars, we are able to obtain storage for about half the commercial rate available through Trans Time".

I note that:

1. Trans Time receives a substantial amount of volunteer labor, and other labor billed at very low cost. Our labor expense for filling capsules amounts to only about 5% of the expenses that we directly cost against long-term storage, making this an insignificant part of the charge.

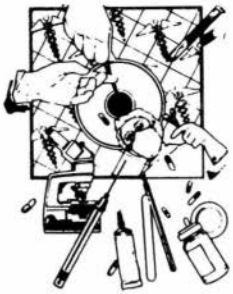
2. Trans Time does own every dewar in its facility used for long term storage of its patients. To the contrary, I understand that the principal dewar used by ALCOR for storage of neuropatients is currently owned by a relative of a suspension patient, to be willed to ALCOR as part of his Donor Fund. In view of this relative's intense involvement with ALCOR, this is probably not a significant problem, but it does make me wonder why you stated the difference between Trans Time and ALCOR's dewar ownership to be the reverse of the actual situation.

In 1983, Trans Time lost $4,246.20 on long term storage of patients, just counting direct costs. This shows the need to increase, not decrease, long term storage charges. Differences between Trans Time and ALCOR's storage charges are not based upon the considerations stated in the CRYONICS article. I believe they are due to more realistic evaluation on Trans Time's part (and even undercharge for) the other necessary overhead accompanying the operation of a cryonics organization that intends to be around for a very long time.

Art Quaife, President
Trans Time, Inc.

"The more important fundamental laws and facts of physical science have all been discovered, and these are now so firmly established that the possibility of their ever being supplanted in consequence of new discoveries is exceedingly remote...Our future discoveries must be looked for in the sixth place of decimals.

—Albert Michelson, Physicist
1894



SCIENCE UPDATES by Thomas Donaldson

BRAIN CONTROL OF CIRCADIAN RHYTHMS

I have felt for a long time that the processes controlling circadian rhythms may have lot to say about aging. As yet no one has drawn any direct connection, but in the interests of increased sophistication among immortalists on this issue, I have reported discoveries in this area for some time. A symposium on the subject of brain control of circadian rhythms recently took place at New Orleans in 1982 under the auspices of FASEB (Federation of American Societies for Experimental Biology), and immortalists might find interest in the results discussed at this symposium.

The major concept coming out of recent work in chronobiology (the biology of time, ie. rhythmic biological behavior) is that of pacemakers. Our individual cells, and many organs, all have their own autonomous rhythms, which they will adopt if cultured in vitro. However in a whole living animal these oscillations never work independently; there are particular oscillators, the pacemakers, which play a coordinating role, forcing all the other rhythms to operate in phase with their own rhythm. Furthermore, in intact animals, these pacemakers will adopt the light and dark cycle of their environment, so long as it does not deviate TOO much from a period of 24 hours (extreme deviations, say 14 days light and 14 days dark, will not be followed).

A further major discovery of the last few years is the identification of at least one of the pacemakers in mammals, which turns out to be two paired brain regions known as the suprachiasmatic nuclei, the SCN. Much of the symposium dealt with studies of this brain region and its response to drug and surgical treatment. However the meeting also saw very strong evidence that our understanding of even the gross anatomy of daily rhythms wasn't yet complete: there is at least one other major pacemaker which hasn't yet even got an anatomical location.

Gerard Groos et al, from the National Institute of Mental Health, NIH, summarized the electrical and biochemical behavior of the suprachiasmatic nuclei (FED PROC 42 (1983) 2790). The strongest evidence that the SCN control biological rhythms comes from surgical experiments in which they are removed and subsequent hormonal cycling in other organs and tissues will stop. Furthermore electrical currents to the SCN will change the rhythm in other brain regions. A good deal of subsequent work has developed information about their biochemistry and anatomy. Nerves run from the eyes to the SCN; these nerves respond only to gross differences in the level of lighting, and the information they bring sets the phase and the length of period for the daily oscillation of the SCN. As yet, complete proof that the SCN will engage in autonomous cycling doesn't exist. However the SCN will show daily rhythms of electrical activity even if isolated from

the rest of the brain by severing of nervous connections. Other brain regions will not do so. Furthermore, individual cells cultured from the SCN will show a rhythm in culture. Biochemical changes such as cycles in glucose consumption will also take place rhythmically in the SCN. However a determined skeptic might still claim that the rhythms of the SCN are themselves perhaps controlled by other hormonal or temperature rhythms.

For aging research one of the most interesting recent results comes from work on how these daily rhythms relate to several important neurotransmitters. It turns out in rats that inhibiting the synthesis of serotonin either by giving chlorophenylalanine or by feeding the animals a diet deficient in the amino acid TRYPTOPHAN will cause a disappearance of circadian rhythms during the time that serotonin levels are reduced (J Lanior et al EXP BRAIN RES 41 (1981) 346-357). This effect should remind us of Segall's work, in which tryptophan deprivation seems to slow the aging of rats. Groos et al feel that the SCN does not need serotonin to generate a rhythm, but rather that serotonin plays an important role in the transmission of the rhythm once it exists. Injection of serotonin into the SCN will not affect period of rhythmic cycling, either in animals isolated from periodic cues or in animals living normally.

Considerable conventional medical interest in rhythmic cycling and its chemistry stems from an interesting fact, which certainly can't make us immortal but might interest us nevertheless. It turns out that considerable evidenceof a circumstantial kind, reviewed by TA Wehr et al (FED PROC 42 (1983) 2809), suggests a strong link between disturbances of daily cycling and manic-depressive illness. Patients with this problem generally pass through periods of severe depression, lasting for days. They will then go through one or a few sleepless nights, after which they will recover from their depression and may even become manic. In their depressed phase these patients, besides being depressed, also sleep a lot. In their manic phase they become optimistic and grandiose, and need less sleep than normal. Often this mania followed by depression repeats quite regularly, in a yearly cycle; this itself would be suggestive. The basic hypothesis behind this work comes from the idea that manic depressive patients have rhythms which are out of synchronization, and the periods of depression and mania happen because of beats in the two rhythms.

It turns out that manic-depressive patients do have quite definite abnormalities in their rhythmic cycling. One manic depressive patient, taken as typical, spent 19 days isolated from any external cues about the passage of time. Her cycling took longer than normal to become established and when it did, showed signs of at least one rhythm markedly less than the normal 24 hour cycle. Furthermore, 4 out of 7 manic depressive patients in one experiment could terminate their depressions by waking up, and staying awake, at 1:00 o'clock in the morning. This caused a remission of depression which lasted for one week or more. Finally, lithium carbonate, which helps suppress the

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GLYCOPROTEINS AND MEMORY

Besides the normal glucose, sucrose, and fructose sugars which we all know and some of us love, there are many other sugars which play important metabolic roles. One of these, fucose, may play a role in memory storage itself, not directly but rather in combination with a protein. Such combinations of sugars and proteins are called glycoproteins, and evidence exists that glycoproteins consisting of a protein combination with the sugar fucose, may play some role in memory storage (Rose, SPR et al J NEUROCHEMISTRY 34 (1980) 1000-1006). Furthermore, fucose itself may improve memory in animals.

A recent experiment by B Lossner and SPR Rose, reported in J of NEUROCHEMISTRY (41 (1983) 1357-1363) give us some interesting information on how fucose and its associated glycoprotein may be involved in memory. Besides strengthening the case that they are involved, this new data points us a bit more toward an understanding of exactly how.

One of the more interesting facts about these fucose glycoproteins consists of their location in the cells: they concentrate in the synapses, the points of connection between different nerve cells. They may.therefore play some role in regulating the transmission of nerve messages; such regulation, in one form, must equal learning itself. Furthermore, in earlier work Rose and others have shown that brains produce these glycoproteins as part of learning.

In this most recent paper, Lossner and Rose studied the metabolic processes by which brain cells produce glycoproteins and how these processes take place preferentially in parts of the brain we would expect to be involved in learning. They studied one particular form of learning in chicks, learning not to peck at a bitter-tasting bead. Chicks will learn not to peck in only one trial, and essentially Lossner and Rose trained chicks in this way and then studied the levels of enzymes involved in making fucose glycoproteins in the brains of their chicks after they had learned.

Two major enzymes work together to make the fucose glycoproteins. One of them, called fucokinase, makes fucose phosphate from fucose. The second, called fucosyltransferase, takes this fucose phosphate and transfers the fucose to the protein. Earlier experiments have shown that when learning occurs, the levels of fucosyltransferase did not change (RD Burgoyne, SPR Rose J NEUROCHEM 34 (1980) 510-517). This latest experiment shows that levels of fucokinase will quite definitely change with learning.

Furthermore, it turns out that this increase in the levels of fucokinase doesn't happen globally all over the brains of the chicks, but instead only in particular brain regions which should be involved in formation of a new memory. In the chicks this is

the right base of the forebrain. Control chicks who did not learn showed no such localized increase, and the fact of such a location provides particularly cogent evidence for a relation between fucose metabolism and learning.

Rose et al speculate that changes in magnesium or calcium ions in the brain cells may cause changes in the level of fucokinase. A change in the level of fucokinase might then go on to produce fucose glycoproteins, a more permanent form of memory. This might connect these changes in glycoproteins with other suggestions that changes in calcium levels are the "first step" in learning. Presently of course this is only speculation; this work by Rose and his other coworkers does, however, definitely create a case that fucose and glycoproteins containing fucose should receive close attention for their structure and physiological role in memory and recall.

(Continued from page 9.)

mania-depression cycling, will lengthen the period of daily rhythms both in people and in experimental animals. Several similar kinds of evidence also suggest a close relation between deranged cycling manic-depression.

Finally a paper by Martin Moore-Ede at Harvard Medical School summarizes the evidence that the story of circadian rhythms is far from over: there is at least one other major pacemaker different from the SCN which we still haven't identified. He shows that there are two different classes of rhythmic cycling, one of which is controlled by the SCN and the other not. One of these groups (controlled by the SCN) involves the sleep-waking cycle, growth hormone cycles, calcium excretion, and slow-wave sleep. The other involves rhythms of core body temperature, blood cortisone concentration, REM sleep, and potassium excretion; the SCN does not control these rhythms. The reason for believing in two independent pacemakers comes from studies in which people are isolated from all time cues for periods of several months. If isolated for long enough, these two rhythms will go out of phase with one another. Furthermore, hamsters, after losing their SCN by surgery, will still retain body-temperature rhythms. Similar studies in monkeys show that removing the SCN will not remove the daily rhythm in blood cortisone concentration.

Looked at objectively, scientific study of our body's rhythmic system has a long way to go. No direct proof of a relation with aging yet exists. However it still stands to reason that our bodies must have some means to measure time, both to tell us when to go into puberty and to tell us when to become old. It therefore stands to reason that studies of clocking and body clocks should have a high priority for aging research.

WHAT YOU CAN DO—PART III

by Mike Darwin

(About a year ago, Thomas Donaldson, President of the Cryonics Society of Australia authored an excellent two part article on "What You Can Do" if you live a good distance from a cryonics organization. Dr. Donaldson's suggestions were along the line of how to improve your position if you were simply incapable of relocating. Now, with apologies to Dr. Donaldson, I herewith offer Part-III of "What You Can Do" for all of you folks out there who didn't pay any attention to the sage advice offered in Parts I & II...or who tried it and didn't like it.)

So, you're sitting there in New Prague, Minnesota; Sayville, Long Island or God forbid, Cleveland, Ohio, and you're wondering WHAT YOU CAN DO. You're not impressed with the idea of stacking up bottles of water for injection near the fireplace (to keep them from freezing) and waiting for help to arrive from Sunny Southern California, and you just don't have it in you to start a cryonics society in a land where people can find out everything they want to know about being frozen merely by waiting for a bus. You know you're not getting any younger and you're ready to take up a cudgel and fight—or at least stand in the shadow of someone who has taken up a cudgel--but you're not sure how to go about finding out what you can do. Well, then this article is for you!

Whether you are 17 or 70, this article will try to tell you not only WHY you should consider moving to Southern California (or even Northern California, though naturally in this article the case for the South will be stronger than the North—let'em write their own WHAT YOU CAN DO PART IV) but how to go about it. We'll try to cover all the bases, and we'll even include a section on the trials and tribulations of IMMIGRATING to the United States (yes, contrary to what you may have heard Los Angeles is still in the U.S.) for those people unlucky enough not to have been born here.

First, the WHYs of considering a move. While it is true, as Donaldson has pointed out, that there are many things you can do to improve your chances if you a live a good distance away from any active cryonics group, it is also true that there is NO SUBSTITUTE for being close to help when you need it. The point has been made many times before (with much justice) that you can always move later, say when your risks of needing help start to go up sharply. Being 112 years old would be a good example of a time to consider moving in short order if you lived in New Prague, Sayville or Paris. Having a terminal illness would be another case where a quick move might be given careful consideration. Unfortunately, being terminally ill or 112 do not really predispose one to good judgement, and in any event just the practical aspects of such a sweeping decision (selling a home, packing possessions, leaving friends and supportive others) when already compromised can be formidable—perhaps too formidable. How much better to make those practical and life wrenching decisions while in good health and with a clear mind. Also, there's the point, and it is a very good one, that you may JUST NOT KNOW when check out time is. How embarrassing to find yourself suddenly and inconveniently dead thousands of miles away from the nearest perfusion facility. Not to mention dangerous.

Of course, moving NOW will make a lot more sense to you if you're not happy now. It can get pretty lonely in Buffalo or Indianapolis with no other

cryonicists around. It can get pretty boring too. This is generally not a problem for those who make the decision to get into the thick of a life with cryonics. If its adventure and hard work you want, well, we've got plenty of both. So, if life is going nowhere for you and three feet of snow, or even a little coating of frost (for those who live in Virginia) has got you down, give Sunny Southern California some long and careful consideration.

So far we have just discussed ways in which the Big Move might be directly beneficial to you. Now perhaps we should touch on how it may be directly beneficial to cryonics and indirectly beneficial to you as well. For a long time cryonics has suffered from undercapitalization. Not enough dollars, not enough good brains, not enough strong arms and backs. This has led some of the good brains, strong arms and tough backs to get more than their share of the ADVENTURE I spoke of earlier with the net effect being that they have run off to mountains, plains and islands in order to take a little rest and recover. We'd like to coax these people back (they know who they are so we'll be polite and not mention names here) and we'd like to do a little something to discourage others from similar behavior. This latter objective is particularly important because if the others (who happen to be the people publishing this magazine) run off too, there might not be anyone around to freeze anyone when we all get to be 112 years old or have something equally fatal happen to us. What all this boils down to is that MORE people working, cooperating and participating means LESS burnout.

But it's more than that. After all, asking you to solve our PROBLEM isn't a very good way to you lure away from all that snow. Perhaps the best thing thing we can do is to point out that we have experienced some growth and that burnout is becoming something less of a problem. What we would like to experience is REAL GROWTH. We would like to get to a point where we have the luxury of having enough people to start doing MORE than just keeping the shop doors open. We'd like to grow, take on new challenges, try new things. To do that we need more talented people who are willing to offer support. That's perhaps the best reason for you to consider coming to Southern California. Who knows--if we get the kind of help we need, it may be that by the time you're 110 we'll have real Suspended Animation. That's how moving might indirectly benefit you. And, if you think about it, maybe that isn't such an indirect benefit after all!

Now, we come to the issue of WHO should move. Hopefully we've gone a long way towards convincing you TO move. Now, perhaps it's time to pause and talk a little about whether you SHOULD move. First of all, OUR requirements. (It may surprise you, but we aren't interested in just anyone, we have some specific qualities in mind). What we are looking for is someone who is above all interested in DOING something, or at least in supporting people who are. Almost anyone with a desire to DO SOMETHING has something to contribute that we're interested in. Naturally, we're more interested in some things than others. If you are a biochemist, if you are good with your hands, if you can speak well in public or operate an electron microscope we are especially interested in you. Obviously being a brain surgeon or just a plain old M.D. wouldn't necessarily disqualify you either.

But even if you're none of the above things, but have a strong desire to help out and a willingness to concentrate your effort, we need you and want you. The world is full of shiftless genius and unused skill. One person of modest abilities and extraordinary will is worth a thousand of the former two types. We know that and hope we never forget it and that you don't either.

If you fit the above "requirements" and you're seriously thinking about moving, then there's a whole lot of things you are going to need to know. We can't cover all of them here, but we can make a start. We can also point out

that we stand ready and willing to help you with the answers to specific questions if you'll just write us and ask.

As to exactly where we are at. We are located in suburban Orange County which is in reality a part of the greater Los Angeles area. It is a very nice area, but rents are rather pricey (I pay a little under $400 a month for a one bedroom apartment). If you are willing to live a little farther away from the laboratory, much more reasonable rents can be found. Also, some of us find Orange County a rather boring place to live (as far as noncryonics activities are concerned) and prefer the racier areas of L.A. such as Hollywood (more on this later). But, regardless of where you live in Los Angeles, the one thing you will need is a CAR or AUTOMOBILE for those of you who have lived in New York City all your life. (I suppose you could scrape by with a motorcyle: but only if you're really crazy!) You will also need to know how to DRIVE your automobile. Some years ago, I arrived in Los Angeles with neither an automobile or any inkling of how to drive one. THIS WAS A MISTAKE. I am trying to save you from a similar BAD EXPERIENCE. Obviously, it is best if you have at least learned the basics of how to drive before arriving in L.A. Without a car you will find yourself PARALYZED in L.A. You will also probably find yourself without a job. It is possible to work and live in L.A. without a car, but it isn't easy, and I don't have the time or two to three hundred pages that would be required to tell you how to go about doing it. LESSON ONE: Get a car, learn how to drive.

The next thing you will need is a JOB. If you are already a well payed professional with highly marketable skills this will probably not be much of a problem. However, if you are fresh out of high school or college and you don't have a degree, or worse still have one in something like Forestry or Marine Biology, you are going to need some advice. If you don't have a marketable skill you need to GET ONE before coming to L.A....or be prepared for a low standard of living and a long struggle working your way up the ladder. To a great extent this is true anywhere you live. You've got to have something to do to earn a living. Keep in mind that all of California and particularly the metropolitan areas such as L.A. and San Francisco are highly cosmopolitan and are thus highly competitive. That can take some getting used to and it can be great fun as well. I cannot begin to tell you how living here has broadened my perspective. I count people from all over the globe as friends and co-workers--Phillippinos, Chinese, Mexicans, Chileans, Swiss...the list goes on and on. This city is the new melting pot and what's better still is that everyone here seems to know it and love it. Certainly there are no prejudices as to friends and food, and if you like to eat the only place in the U.S. that can hold a candle to L.A. in terms of the diversity of good cuisine available is New York City.

If you have a skill, whether it is clerical, professional or day laborer, you will probably have little difficulty in finding a job. Work, for those willing to work, is plentiful. But, as I mentioned earlier, if you don't like the sound of languages you can't understand (or recognize) or you have difficulty in adjusting to people who think or act differently, then you probably shouldn't come to Los Angeles.

If you've got a car, know how to drive, don't have xenophobia, do have a marketable skill, then you're ready to make the move. The question is, exactly where to. After all, L.A. is a BIG place. This is where the next major piece of advice comes in. MAKE AN EXPEDITIONARY TRIP. Sound out the territory. Meet the other cryonicists, look into employment. GET A FEEL FOR THE PLACE. Would you buy a car or rent an apartment without at least seeing them first? This is especially true for people who have not moved around a lot before and doubly, doubly true for people who are living outside the United States and have never been to Los Angeles before. If you live in Indianapolis a move to L.A. (such as

I made) can be enough of a culture shock—and I still speak the same language (well, mostly anyway) and grew up in the "same" culture. If you're considering coming from outside the U.S. it is going to be even more of a shock (yes—everything you've heard about Americans is true: we all own guns, are very aggressive and drive condominiums around). Being prepared for the move will help a lot. Just being sure you like the "style" of Southern California cryonics will count greatly towards building security in a decision to move.

Visiting first will also help you to decide quite specifically where to live. And almost no place has the kind of choices in terms of climates and lifestyles that L.A. has to offer. Here is where my advice will be somewhat limited, because clearly I haven't lived everywhere in Los Angeles. But I do have some feel for the flavors of the city and I'll do the best I can to share it.

If you want to be closely involved with cryonics on a day to day basis, then it will probably have to be North Orange County or someplace within reasonable striking distance of there (keep in mind that to many Angelenos reasonable striking distance means 45 minutes to an hour!). The North Orange County area offers everything from small town ambience (Brea, Yorba Linda, Montclair) to the hustle-bustle of Disneyland which is about 15 minutes away from ALCOR. If you're into the rural scene, we are surrounded by farm land, and on Sunday afternoons horses, from ranches a mile or two up in the hills, amble by (most often accompanied by riders).

If you want more occasional involvement or if you insist on a spicier style of life than Orange County has to offer, then you should consider Los Angeles proper or its "suburbs": Hollywood, North Hollywood, Westwood, Beverly Hills and so on. These are the areas where the action is. Areas where newly released movies open, where gay bars and ladies of the evening are, and where the more culturally exciting and depraved (depending of course on your point of view) sides of life are to be found. L.A. has plenty of good theatre, good symphony, good resteraunts. Housing can even be inexpensive in these areas—though the the crime rate tends to be high. (One advantage of Brea is that it has the lowest crime rate of Orange and L.A. counties combined.) If you LIKE living in Indianapolis, but can't stand the climate, you might consider Long Beach, a city without the rough edges of L.A., but with some of its spicier aspects. Long Beach has a nice ambience about it, lots of things to see and do, and good employment opportunities. It doesn't have the somewhat raw-edged-crazy feel that Hollywood and other parts of L.A. are sometimes given to. There are few bag ladies in Long Beach and fewer still of the people who stand on the street corners carrying on long, animated conversations with thin air. There are NO people like that in Orange County, and so, despite the fact that the good movies open weeks later here, life does have its compensations.

If you want to really get away from it all there is La Crescenta, La Canada and Tujunga to the North, and these little towns (all growing like weeds) nestled in the foothills of the San Gabriel Mountains offer breathtaking views and frequently breathtaking weather—both literally and figuratively. When living there, it is usually better to rent than buy and to store your most precious possessions with Bekins or Mayflower somewhere on safer ground. Of course, for those who really want to get away, there is Mount Baldy (about an hour and fifteen minutes from Cryovita) with its ski areas and jobs (somewhat seasonal) right at your doorstep.

While this capsule summary by no means covers all the ground, it should serve to give you an idea of the incredible diversity available in Southern California. Indeed, it can almost be said that it is IMPOSSIBLE not to find some place which offers everything you're looking for. Snow, sunshine, mountains, beach, desert, forest, small town, big city, conservative or radical, Southern California has something for everyone. Especially cryonicists!

END OF PART I OF PART III OF WHAT YOU CAN DO

REPORT ON THE 20TH ANNUAL MEETING OF THE SOCIETY FOR CRYOBIOLOGY—PART II

In part II of our coverage of the 1983 annual meeting of the Society for Cryobiology, we report on liquid state tissue, organ, and organism preservation.

NON-FROZEN PRESERVATION

At the "Conversazione" evening session, J. Foreman, et. al. of the MRC Medical Cryobiology Group in Cambridge described his group's continuing studies of the reversal of warm ischemic injury using oxygen gaseous perfuson at reduced temperatures. With this technique the gas is delivered through the renal vein rather than the renal artery, accounting for the term "retrograde oxygen persufflation" (ROP). Three of five kidneys damaged by 60 minutes of warm ischemia apparently functioned following ROP for 24 hours. Four of five kidneys functioned after 30 minutes of warm ischemia and 48 hours of ROP. Apparently the technique depends on oxygen, since nitrogen and helium gave nonfunctioning kidneys, and results with air were not as good as results with oxygen. This finding is a little confusing in view of reports from other labs on the ability of redox control at low redox potentials to reverse ischemic injury: ROP presumably uses high redox potentials. Nevertheless, this is a valuable addition to the literature on reversal of ischemic injury.

M. Kallerhoff et. al. (Germany) presented a very interesting poster on the use of a cardioplegic solution (HTK) to protect kidneys from warm and cold ischemia. Dog kidneys were perfused with HTK or with "Euro-Collins" solution (i.e. Collins solution without magnesium sulfate) at 5 degrees centigrade, 15 degrees centigrade, 25 degrees centigrade and 35 degrees centigrade and subjected to ischemia while intrarenal pH was measured. With Euro-Collins, pH fell to 6.4 in 36 hour at 5 degrees centigrade and in 1 hour at 25 degrees centigrade. With HTK, pH was 7.3 after 36 hours at 5 degrees centigrade and 6.8 after 12 hours at 25 degrees centigrade. Kidneys perfused with HTK and kept ischemic at 32 degrees centigrade for up to 130 minutes retained good life support function, whereas Euro-Collins or no perfusion gave dead or non-functioning kidneys after this degree of ischemia at 30 degrees centigrade. The whole question of pH inside organs being stored on ice without perfusion and its relationship to organ deterioration has scarcely been addressed before. Hopefully this study gives organ preservationists another good lead to explore.

A more conventional study of cardioplegic solutions was described by W. Isselhard and co-workers (Germany). They found potassium and calcium to be damaging during ischemia at 37 degrees centigrade. At 27 degrees centigrade, calcium was still damaging but potassium was not. At 17 degrees centigrade, neither calcium or potassium was damaging to the rabbit heart. These trends were based on both biochemical and microscopic analyses. The findings may indicate that cryonics perfusates can have considerable leeway in their electrolyte composition without producing cellular damage, assuming perfusion takes place below 17 degrees centigrade.

P.G. Spieckermann et. al. (Germany) also found that the effect of calcium blockers is reduced below 25 to 28 degrees centigrade, possibly due to a phase change in one of the critical membrane systems that controls calcium action in the heart.

David Pegg, J. Foreman, and K. Rolles found that ischemically damaged rabbit and dog kidneys could be made to regenerate ATP at 10 degrees centigrade, but only if certain metabolic substrates, a high (600 mm Hg) oxygen tension, and a certain a colloid (Haemaccel) were all present. Unfortunately, dog kidneys

damaged by one hour of warm ischemia and then regenerated in this fashion for 2 days still failed to survive after transplantation due to vascular injury.

V.C. Marshall et. al. looked at the effects of glucose on ice-stored rat kidneys preserved with their citrate-based solution. Their data indicated that glucose may damage the center (medulla) of the kidney, even in low (10mM) levels. However, they suggested that this could be due to pH effect, since glucose might be broken down into lactic acid. This explanation is quite possibly correct since Collins solution, which contains high (140 mM) levels of glucose but is well-buffered, is obviously a good solution for ice storage of kidneys. (At higher temperatures, though, glucose-rich solutions are damaging, partly due to enhanced cellular penetration, partly due to increased breakdown into lactic acid. Chemical reaction between the glucose and cellular proteins via the Maillard reaction or "non-enzymatic browning" process may also be involved).

Session 9 the next day contained 2 papers of interest dealing with liquid state organ preservation. In the first, Collins et. al. reported that a study by 5 transplant centers in the U.S. supported the use of high magnesium sulfate concentrations for human kidney storage (Collins solution versus "Euro-Collins"). This finding is not really a new one, but lends added weight to the conclusion that magnesium is beneficial during cold ischemia. Combined with the fact that magnesium can protect brain cells against anoxia (see Cryonics #35, p. 16, June 1983), this study suggests magnesium should be more carefully considered for cryonics perfusates. However, the magnesium effects are small for kidneys, and it is not clear how compatible magnesium is with cryoprotective agents.

The second paper, by R. McCabe et. al. of Australia, indicated that ice storage (no perfusion) or perfusion preservation with cryoprecipitated plasma (at around 10 degrees centigrade) were equally effective for kidney preservation but should not be combined. The damaging effect of combining ischemic preservation (0 degrees centigrade) with perfusion preservation has been found by other investigators to be due to a reaction between the magnesium of Collins solution and the fibrin present in cryoprecipitated plasma, leading to intravascular emboli. The implications for cryonics would be that if we elect to use high magnesium levels in our perfusates, we should not introduce these high levels until most of the blood has been washed out.

Session 12 was devoted entirely to hypothermic preservation of kidneys and livers. The first paper was by I.A. Jacobsen and co-workers (Denmark). Jacobsen has done some outstanding work in cryobiology, especially in the area of rabbit kidney glycerolization and freezing. This current effort involved simple 0 degrees centigrade storage of rabbit kidneys after flushing with either Collins solution or a new solution, LIC, which is based on Fahy's RPS-2 solution. Although no kidneys flushed with Collins solution survived 48 hours of storage, the LIC kidneys did well. Hypertonic citrate solution gave inferior results. These experiments were carried out partly in order to decide upon the best carrier solution for additonal studies of kidney cryoprotection.

Vernon Marshall and colleagues next reported their experiemnts on hypothermic rat kidney preservation. The most important finding was that their citrate-magnesium solution, normally used for ice storage (no perfusion), could also be used for continous perfusion preservation when a colloid (5% albumin) was added. This is a departure from tradition, since usually radically different types of solutions are used for continuous perfusion and ice storage. Marshall et. al actually found the citrate solution to be superior for perfusion compared to the normal extracellular-type perfusate. Similar results of Belzer et. al. using a gluconate-based solution suggest a new direction in organ

preservation.

J. Southard then presented the work of himself, M. Kuniyoshi, and F. Belzer (Madison, Wisconsin). They used the tissue slice technique to look at mitochondrial function, tissue water content, and tissue potassium levels in dog kidneys stored for either 3 or 5 days. The purpose was to see what differed between 3 days, at which time the kidneys are viable, and 5 days, at which time the kidneys are not viable. The mitochondrial assays did not change much between 3 and 5 days, whereas the water and potassium contents did change. They concluded that preservation injury appears to involve increased membrane permeability rather than mitochondrial injury.

G. Pavlock of the same group then reported on studies of the role of lysosomes in kidney deterioration. Two of three lysosomal enzymes increased considerably between days 3 and 5, suggesting that lysosome activity may limit preservation times.

A third paper from the same lab was submitted by J. Noon. He reported that ATP levels of rabbit kidneys previously subjectd to three hours of warm ischemia could be regenerated using a normothermic perfusate containing 10 mM adenosine! If the rabbits were pretreated with chlorpromazine (3.5 mg/kg) and the adenosine normothermic perfusate used as well, ATP could be regenerated in kidneys subjected to 3 1/2 hours of warm ischemia! ATP regeneration could similarly be achieved in ice-stored kidneys after 3 days of preservation. These are spectacular results and suggest that mitochondrial damage is not the limiting factor in warm or cold ischemic injury.

G.L. Cohen et. al. reported that the octanoate (fatty acid) stabilizer present in plasma protein fraction may be damaging to kidneys.

J. Lunec, B. Fuller, and C. Green found that re-exposing either kidney tissue or whole kidneys to oxygen following previous ischemia induced lipid peroxidation. This finding should further help to improve death (i.e. warm ischemic injury) reversal technology.

The next paper (B.G. Rijkmans, W.A. Buurman, and G. Kootstra, of the Netherlands) reported a significant step toward practical application of the group's earlier finding that kidney preservation times can be doubled if the kidney is perfused by a host animal for 2 to 3 hours after 3 days of cold perfusion and prior to an additional 3 days of hypothermic perfusion. The new step was to substitute a heart-lung machine and collected blood for the host animal. Apparently a 3 hour normothermic blood perfusion with a heart-lung machine is sufficient to reverse 3 days of preservation injury. Unfortunately, if this technique is successfully extended from 6 days to 12 days or 24 days or beyond, interest in organ cryopreservation could disappear!

The final paper of interest in this session was that of M. Ukikusa and T.S. Lie, who found a relationship between the quality of liver preservation and pH. Plain Ringer's solution could not preserve livers longer than 4 1/2 hours, whereas if the pH was adjusted to 10.0 before flushing, preservation could be extended to 9 to 12 hours. This results is hard to explain, however, because Ringer's has no pH buffering capacity, so the initial pH should be basically immaterial. Collins solution was more effective at maintaining tissue levels of high energy compounds (ATP and ADP) when it pH was 9.0 rather than 7.4. This is more sensible, since Collins solution has significant buffering capacity. Certainly research on the role of pH at 0 degrees centigrade is valuable for cryonics, and it is good to know that very alkaline pHs are not apparently damaging.

Session 13 was a symposium on Comparative Aspects of Hypothermia and Hibernation. However, it held little information of practical value. The fact that hibernators' organs function better in the cold than do organs from non-

hibernators was demonstrated over and over, but few clues turned up as to how to make non-hibernators more like hibernators. The most provocative results were those of Peter R. Oeltgen and Wilma A. Spurrier (University of Kentucky and Loyola University Medical Center, respectively) concerning the effects of a putative "hibernation induction trigger" (HIT) which Oeltgen has been studying for a number of years. When HIT isolated from hibernating woodchucks is injected either intravenously or into the cerebral ventricles of monkeys, heart rate falls 46% for as long as 8 hours afterwards, body temperature falls as much as 3 degrees centigrade and remains low for 3 to 5 hours, renal filtration rate falls 50% and the animals fall over and enter a state resembling anesthesia. Non-hibernating (summer) woodchucks of the same species do no seem to have this HIT, since the same fraction of their plasma does not produce these effects in monkeys. HIT also causes a severe suppression of appetite lasting 1 to 3 weeks after the injection, yet despite the reduced food intake, these animals do not lose weight. This must indicate a greatly reduced metabolic rate. Although the latter property could make HIT an intriguing compound to screen for anti-aging effects, there is no direct evidence as yet that HIT can actually improve the tolerance of non-hibernators' organs or bodies to hypothermic storage.

Perhaps an even better clue than the HIT was provided by X. J. Musacchia (University of Louisville), who found that hypothermic hamsters, which are normally stable for only 6 to 20 hours at 7 degrees centigrade, can be made to survive for 4 days in the hypothermic (non-hibernating) state simply by infusing them with glucose. It seems that hypothermia in contrast to hibernation, causes a 95% drop in blood glucose level. Pretreatment with cortisone acetate (4 mg intraperitoneally daily for five days) also improved survival.

In session 15, on hibernation and hypothermia, Colin J. Green and B.J. Fuller et. al. reported on their work with isolated organs from hibernating ground squirrels. Kidneys flushed with hypertonic citrate solutions could be stored for 10 days if taken from hibernating animals, versus 3 days if taken from non-hibernating animals.

Another abstract published as part of session 15 was that of Pava and Vojin Popovic, J.C. Beggs, and E. C. Burdette. Popvic is know to at least some cryonicists for his work on the freezing of newborn ground squirrels and for his book on hypothermia in which he predicted the successful freezing of whole mammals. E.C. Burdette has been instrumental in the development of microwave thawing techniques for frozen kidneys and frozen blood cells. In this study the two applied local microwavee rewarming of the heart in situ in hypothermic adult rats previously cooled to either 10 degrees centigrade or 8 degrees centigrade. Microwave warming raised the survival rate from 50% at 10 degrees centigrade without microwaves to 66% with microwaves at the same temperature. More impressively, the survival rate at 8 degrees centigrade went from 0% without microwaves to 50% with microwave heating. The reasons for these results are not clear.

This concludes part II of our coverage. Part III will continue next issue with fundamental aspects of cryobiology and miscellaneous points of interest.

"Imperious Caesar, dead and turned to clay,
Might stop a hole to keep the wind away.
Oh, that that earth which kept the world in awe
Should patch a wall to expell the winter's flaw!

--Shakespeare
Hamelt

Perfusion: Acute Vascular Obstructions and Cold Agglutinins

by Jerry D. Leaf

Introduction

"Perfusion" refers to the delivery of substances to the tissue through the vascular system by a pumping action. The criteria for determining the adequacy of a particular perfusion will depend on the specific goal of the perfusion protocol. In perfusion of suspension patients the goal is delivery of cryoprotective agents to every cell that is to be preserved and to provide necessary metabolic support during this procedure. A necessary condition for any successful perfusion is that the perfusate flow through the vascular system of every tissue. Some of the factors influencing perfusion and delivery to the tissues are: physiological status, peripheral vascular resistance, perfusate composition, viscosity, pressure, type and quantity of flow. The principles are the same, whether the perfusion is with whole blood, containing formed elements, or with specially designed asanguineous fluids, as are used for cryoprotective perfusions. This paper will discuss patency of the vascular system, in terms of potential obstructions to adequate perfusion. In particular, the perfusion for cryonic suspesion often entails special conditions that further complicate and influence the degree of cryoprotection. Acute and chronic disease, including aging, ischemic injury, reperfusion injury, pharmaceuticals and prolonged exposure to toxic concentrations of cryoprotective agents combine to produce variable results. The recognition and control of as many of these factors as possible will lead to improved results.

Acute Vascular Obstruction

Obstruction to flow in the vascular sustem can have many origins, both chronic and acute, however, the most common that will be seen in suspension patients are of the kinds listed in Figure 1. As is usually the case, preventative actions are the most important measures that can be taken, and sometimes the only means of dealing with acute vascular obstruction.

Acute Mechanisms of Vascular Obstruction

PARTICULATE EMBOLI

THROMBOSIS

ROULEAUX

AGGLUTINATION

AIR EMBOLUS

FAT EMBOLUS

FIGURE 1

Gases, as well as solids, can obstruct flow in the vascular system. Most gaseous emboli can be avoided by meticulous attention to established principles of intravenous administration , invasive arterial pressure monitoring, surgical cannulation techniques, proper priming of extracorporeal circuits and the safe operation of extracorporeal perfusion pumps. The most devastating injury can be produced by massive air embolism from extracorporeal pumping. These accidents are usually caused by allowing an arterial reservoir to run dry and can only be prevented by having a perfusionist constantly monitor fluid levels. No combinations of level monitors and automatic pump shutoff controls has proven failsafe. At Cryovita Laboratories, we have never pumped air into a patient, which is a record that will only be maintained by constant vigilance. Among extracorporeal technologists there is a saying; "There are only two kinds of perfusionists, those who have pumped air, and those who will." If you are to avoid this kind of tragedy, never allow a pump to be operated without 100% attendance by qualified personnel.

Solid emboli will have different forms, depending on their origin. Solid

particles (particulate emboli) may come from an unfiltered extracorporeal circuit as plastic debris is shed from oxygenators or other equipment in the circuit. Adequate arterial line filtration, using 20 to 40 micron screen filters, will minimize particulate emboli from this source. Unfiltered or improperly filtered perfusate is another potential source of particulate emboli. Pre-filtration of perfusate with 0.2 micron screen filters will virtually eliminate perfusate sources of particulate embolization.

Fat emboli from bone marrow are a more rare complication. Globules of fat from cut or traumatized bone can migrate into the vascular system. The possibility of fat embolism can be minimized by placing surgical bone wax on the cut surfaces of bone, as when a median sternotomy has been performed.

Thrombosis (intravascular blood coagulation) can be a major source of acute vascular obstruction. Primary cause of thrombosis in suspension patients is likely to be stasis of blood flow due to cardiac arrest, or prolonged low blood flow during transport, without adequate anticoagulation. If patients are transported by commercial air carrier, without total body washout (TBW), and the clotting mechanism is intact, massive intravascular coagulation can be expected. Even though heparin is given, if hypothermia is not complete, metabolic acidosis will cause blood pH to fall and heparin will begin to be deactivated at a pH of 7.10. This is another reason TBW should be carried out before transport by commercial air carrier. Coagulation can be avoided during prolonged low flow states, as may occur during heart-lung resuscitation (HLR) transport, by using buffers to control pH; by maintaining adequate heparinization; by hemodiluting with dextran 40 to improve microcirculation and by employing hypothermia. Hypothermia will help control pH in two ways; by lowering metabolic requirements, i.e. reducing lactic acid production; and by increasing pH in accordance with the Rosenthal Factor, 0.015 pH units per degree centigrade below 37 degrees centigrade. (1) All of the risk factors mentioned above can be optimally controlled by transporting patients with cardio-pulmonary support on portable ECMO (mobile heart-lung machine with Extracorporeal Membrane Oxygenation), so that adequate perfusion can be achieved over a greater range of temperatures.

Rouleaux Formation

Red blood cell (RBC) aggregation, not involving coagulation mechanisms, can also become a significant factor influencing perfusion. The two kinds of aggregation likely to be seen in suspension patients are rouleaux formation and cold agglutination. Figure 2 shows the difference in appearance and form of these two kinds of aggregation. Rouleaux represents less of an obstruction to blood flow (2), in terms of its ease of disaggregation. Rouleaux formation may occur in circumstances when increased viscosity takes place, as in hypothermia and low flow states (3), from macromolecular bridging (4), from exposure to media with a high dielectric constant (5), and pathologic changes in RBC surface electrical potential.

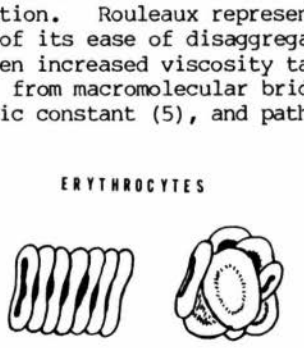

FIGURE 2

Conditions that suspension patients are exposed to are favorable to rouleaux formation. HLR transport creates low flow states, because the technique of sternal compression is unable to produce adequate pressure and flow. Surface cooling, a necessary compensation for HLR inadequacy, increases blood viscosity, further contributing to low flow in peripheral areas. Inadequate oxygen delivery, hypoxia, and consequent lowered blood pH also affect the internal viscosity of the RBC and, therefore, the general viscosity of the blood. (6) RBC resistance to plastic deformation further hinders ease of movement through capillaries because of rouleaux formation.

Macromolecular bridging (Figure 3) can be caused in low flow states by endogenous substances, such as fibrinogen, or by addition of polymers, as plasma expanders, and colloid in base perfusate for total body washout. Polymers, such as dextrans, should be low molecular weight (average molecular weight 40,000), when used in base perfusate or when given for volume replacement during transport. Hespan (average molecular weight 1,000,000) or Rheomacrodex 70 (average molecular weight 70,000) are not ideal I.V. plasma expanders for this reason.

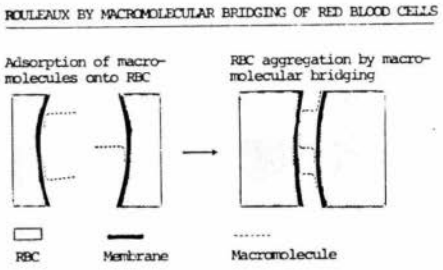

FIGURE 3

Red blood cells have electrically charged membrane surfaces that are predominantly negative in potential. Because of this, under normal conditions, the red cells repulse each other. This electrical field or zeta potential, as it is called, can be altered by pathologic states, exposure to substances having a high dielectric constant and by large anisometric molecules. Therefore, it is inappropriate to use cryoprotective agent, such as dimethyl sulfoxide, in total body washout perfusate, due to the high dielectric constant. This is contrary to past suggestions. Another past practice was the use of polyvinylpyrolidone (PVP) in base perfusate. Large anisometric molecules, such as PVP, can reduce the zeta potential and contribute to blood sludging. (8)

Red blood cell aggregation by rouleaux formation is sensitive to shear rate. (9) Increasing perfusion pressure can cause disaggregation of rouleaux formation by increasing shear. The hemodilution from properly formulated flush solutions for total body washout and appropriate volume expanders for transport protocols will decrease blood viscosity (10) and lower the shear rate needed to increase flow. Moderation in the use of surface hypothermia, which will be discussed further in the next section, will also help minimize problems from rouleaux formation.

Agglutination

Agglutination is characterized by irregular clumps of red blood cells (11), as shown in Figure 2. Cold agglutination is of particular concern for suspension patients due to the various kinds of exposure to hypothermia, during both transport and perfusion protocols. A distinction between "warm" and "cold" agglutination diseases has been made in the past, however, this is less distinct today because of improved testing techniques. Virtually all human red blood cells will show cold agglutination at temperatures between 0 to 5 degrees

centigrade due to the normal presence of antibodies. (12) Our greatest concern will be the cold agglutinins that have a temperature range, or thermal amplitude, between 5 and 15 degrees centigrade, which are the most common. In rarer cases the thermal amplitude can range as high as 31 or 32 degrees centigrade.

The most reactive forms of cold agglutination result in hemolysis of the red cells upon rewarming to 37 degrees centigrade. The result in clinical patients is anemia. The two antibodies usually responsible for cold agglutination are IgM and IgG (13), and represent autoimmune responses to disease or reaction to the presence of drugs. The prevalence of cold agglutination above normal thermal amplitudes is also associated with aging (50+ years).(14) Figure 4 lists some diease states that increase the presence of cold agglutinins.(15) Other diseases associated with pathologic cold agglutination include: rheumatoid arthritis, ulcerative colitis, infections involving viruses, such as cytomegalovirus (CMV) and Epstein-Barr, and clostridia and E. coli infections. Because most suspension patients are over 50 years of age, and considering their likelihood of having a disease and subsequent exposure to medications, it is highly likely they have an increase of cold agglutinins. Figure 5 lists some of the pharmacological agents that affect the level of serum cold agglutinins. (17,18) These drugs represent only a few of the most reactive. A search done for this author by the Drug Information Service at the UCLA Medical Center revealed that the only known antibiotic to be exempt from increasing cold aggutinins in Erythromycin.

Diseases Increase Cold Agglutinins

PNEUMONIA

LYMPHOMA

MONONUCLEOSIS

MEASLES

ARTHRITIS

CYTOMEGALOVIRUS

AGING 50+

FIGURE 4

DRUGS AFFECTING AGGLUTINATION

DECREASE

DEXTRAN

METHYLPREDNISOLONE

INCREASE

CEPHALOTHIN

METHYLDOPA

PENICILLIN

FIGURE 5

The most common laboratory test for cold agglutinins is the Coombs test. Figure 6 shows characteristics from the Coombs test, in which the titer is a measure of cold agglutinin reactivity. However, neither the Coombs test nor its variations are practical for suspension patients, due to long incubation times and other difficulties of accurately performing the tests. Therefore, this author can offer no suggestion for field or laboratory quantifications of cold agglutinins in suspension patients. In those cases of pathologic cold agglutinins, in which rewarming the red cells to 37 degrees centigrade will result in notable hemolysis, a sample can be cooled to 0 degrees centigrade for at least 30 minutes and rewarmed to 37 degrees centigrade. An observation of visibly increased plasma free hemoglobin in such a sample would be a positive indication of a significant presence of cold agglutinins, assuming other factors had not influenced red cell fragility. This is not an assumption that could be made about blood samples from a patient transported long distances by commercial air carrier, packed in ice. Physiologic cold agglutinins are different in as much as they do not cause red cell hemolysis upon rewarming to 37 degrees centigrade. In this case, the red cells simply disaggregate upon rewarming. Since high titers of physiologic cold agglutinins would not be readily apparent with the simple cooling/rewarming test mentioned above, even negative plasma

free hemoglobin would not positively indicate an absence of cold agglutinins with abnormally high thermal amplitudes.

SEROLOGIC CHARACTERISTIS OF COLD AGGLUTIINS

Laboratory Finding	Physiologic Cold Aglutinins	Pathologic Cold Aglutinins
Titer	$\leq$ 16	$>$ 256
Thermal Range	4 deg. C	Above 16 deg. C
Direct Coombs Test	Negative	Positive

FIGURE 6

Strategies for Dealing with Cold Agglutinins

The impracticality of making a definitive diagnosis of cold agglutination thermal amplitude for suspension patients, before the application of hypothermia, calls for a more generalized approach to dealing with the problem.

We can, when applicable, use the two pharmacological agents methylprednisolone (Solu-Medrol) and low molecular weight dextran* (Rheomacrodex 40) to decrease cold agglutination. Both of these agents are already recommended by Cryovita Laboratories as part of its transport protocol.(19) Erthromycin should be used as an antibiotic, since it is the only known one that does not increase cold agglutinins. Erthromycin is a good broad spectrum antibiotic, having effectiveness against both gram positive and gram negative bacteria. In most cases, the elapsed time required for transport, perfusion and cool down to sub-zero temperature would be adequate time for endogenous bacterial growth, therefore, standard medical pre-operative antibiotic regimens are called for, as a prophylactic measure.

The use of hypothermia should be modified from past practices. Previously, suspension patients have been covered with ice packs, with direct application to the body surface. The temperature of skin in direct contact with ice packs will easily be in the 0 to 4 degrees centigrade range, causing cold agglutination of blood. Further, hypothermia complicates peripheral blood flow by increasing blood viscosity and causing cyclic vasodilation and vasoconstriction.(20,21) With extended HLR transport, as core body temperatures fall, deeper subcutaneous tissue and significant portions of the extremities will achieve deep hypothermia. Agglutinated red cells have been observed in peripheral vessels of suspension patients after surface cooling with ice packs. Acute vascular obstruction from cold agglutinated red cells have undoubtedly influenced the flow of cryoprotective perfusates into extremities and peripheral tissues. The problem is increased when patients are packed in ice to be transported by commercial air carrier, without total body washout, resulting in core body temperatures of 3 to 5 degrees centigrade.

Most cold agglutination can be avoided by keeping tissue temperatures above 16 degrees centigrade. When patients are surface cooled for HLR transport, direct skin contact by ice packs must be avoided. The preferred method of surface cooling is by water-alcohol circulated cooling blanket. If ice packs are used, then insulation must be interposed between the patient's skin and the

*Dextrans are incompatible with dimethyl sulfoxide and should not be used if the perfusion protocol calls for dimethylsulfoxide as the cryoprotective agent.

ice pack to avoid lowering the skin temperature below 16 degrees centigrade. If patient transport is by ECMO (extracorporeal membrane oxygenation) with mobile heart-lung machine, the blood temperature is adjusted by a heat exchanger in the extracorporeal circuit. Regardless of the method used to induce hypothermia, cold agglutination can be minimized by maintaining tissue temperature above 16 degrees centigrade, except in the more severe cases of pathological cold agglutinins.

The ideal temperature during transport, when oxidative metabolism is being supported by blood circulation, depends on the percentage of basal requirements that can be supplied by the particular kind of circulation support being used for transport. If 50% of the normothermic (37 degrees centigrade) oxygenation and blood flow requirements can be met, as might be expected with improved HLR transport techniques using diaphragm restraint, then the ideal temperature should be 27 degrees centrigrade, a temperature at which the metabolic requirements are reduced by 50%, according to the Q-10 rule. An ECMO system of transport could approach metabolic support at 37 degrees centigrade, therefore, significant hypothermia and cold agglutination can be completely avoided with this technique of transport.

Every suspension patient requiring transport by commercial air carrier, packed in ice, should undergo total body washout with an appropriate intracellular-type flush solution. The alternative for these patients is rewarming to a temperature at which their particular cold agglutination will disaggregate, during support with a heart-lung machine. This is not without great danger of exacerbating an already ischemically injured body with an even greater reperfusion injury.

Finally, every suspension patient should undergo total body washout with oxygenated perfusates in a temperature range of 17 to 20 degrees centigrade, to optimize considerations of both cold agglutination and metabolic requirements. After TBW, perfusate temperature can be reduced in accordance with the requirements for minimizing cryoprotective agent (CPA) toxicity and maximizing equilibration of CPA. Figure 7 is a schematic summary of the considerations for transport and TBW.

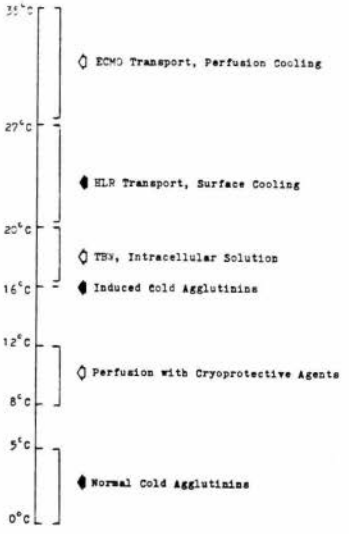

FIGURE 7

Like so many other improvements in cryoprotective perfusion protocols for humans, the observation that led to an understanding of the problem of cold agglutination in suspension patients was made during an actual suspension procedure. The attendance of experienced and trained observers at suspensions is a key factor in the continued advancement of suspension protocols. To this end, I encourage all cryonics organizations to make

arrangements to have observers with medical and cryobiological expertise attend their suspension procedures.

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