CRYONICS

ISSUE #10 MAY 1981

Selected Contents:

CRYONICS is the newsletter of the Institute for Advanced Biological Studies, Inc. and the Alcor Life Extension Foundation. Published monthly. Free to members of IABS and Alcor. Individual subscriptions $8.00 per year. Group rates available upon request. Please address all editorial correspondence to IABS, Inc., 2901 North Pennsylvania, Indianapolis, IN 46205, or phone (317) 924-9886 or (317) 359-7260. Alcor may be contacted at P.O. Box 312, Glendale, CA 91209, (213) 956-6042.

CRYONICS #10 May, 1981

EDITORIAL MATTERS

This month IABS is proud to welcome Alcor Life Extension Foundation as a co-sponsor of this newsletter. We will begin printing Alcor news items in the next issue. Please continue to send donations and editorial material to the IABS address until further notice. Subscriptions to this newsletter may be obtained through a membership in IABS or a donation of at least $8 to IABS or through membership in Alcor. (There is no increase for individuals who have already subscribed at the introductory $5 rate.) Other organizations wishing group rates should contact IABS.

Michael Darwin and Stephen Bridge are co-editors of this publication. Submissions should be camera-ready, typed with a dark ribbon on 8½" x 11" paper. Pica type (that's the large size, folks) is preferred. We reserve the right to reject illiterate or incoherent material, but it is our intention to print any clearly written opinions or news on life extension -- with strong emphasis on cryonics. If you wish us to reprint a letter from you, please include a separate "permission to reprint" statement. Criticism is as welcome as support and will be given equal attention.

The editors are grateful for the many donations and words of encouragement we have received from our readers. However, we are still not up to the volume of copies necessary for us to achieve great reductions in printing costs and postal costs. Therefore, we have been forced to go to this partially-reduced format this month, to allow us to print the large amount of material we had. A number of readers have told us that the content is more important to them than the format, so we hope this change will not inconvenience you. Your comments will be appreciated.

We also welcome as a regular contributor Thomas Donaldson, PhD, who will supply science updates, plus occasional book reviews, opinions and articles.

APOLOGY

"Stephen, I think you should apologize to our readers for your over-eagerness in stating that "The Cost of Cryonics" would be ready for this issue."

"Yes, Michael, you're absolutely right. Readers, writing that article has been like walking through a swamp with a Sousaphone. We hope to untangle the situation enough to publish our conclusions within the next few months."

IABS ANNUAL MEETING

The 1981 annual meeting of the Institute for Advanced Biological Studies will be held on July 12 at 7:00 at the IABS offices, 2901 N. Pennsylvania, Indianapolis. Attendance is limited to current members or those who pay their membership by the time of the meeting. Associate members may attend but are not eligible to vote. We hope all Indianapolis area members will be able to attend this last local meeting before IABS moves its offices to California.

CRYOBIOLOGISTS TO RECEIVE CRYONICS

Beginning with this issue, selected cryobiologists and research centers will begin receiving CRYONICS free of charge. It is our hope that exposing professional cryobiologists to cryonics will at least give them a more rational basis for criticizing us. In the past many cryobiologists have viewed us as shady, impossible-to-locate individuals who reside in the woodwork of the lunatic fringe. It is not our intention to proselytize or convert. We are well aware of the futility of such an effort. Rather, we wish to inform while conveying the message that we are out here and have nothing to hide. We invite comments, criticisms and research information for publication from cryobiologists who may read CRYONICS.

IABS ACQUIRES CAGES

IABS recently acquired two racks of stainless steel mouse cages with automatic watering system, as well as one rack of rabbit cages. The mouse cages are capable of holding 400 animals and are ideal for lifespan or drug toxicity studies. The mouse cages are apparently virtually unused, are of a late model and were purchased for a little over scrap stainless steel prices. The rabbit cages are of galvanized steel construction with stainless steel dropping pans and an automatic watering system. The rabbit cages are capable of holding 12 animals and, while used, are in excellent condition.

These items represent a valuable addition to IABS research capabilities. We are grateful for the financial and personal help received from Mike Darwin and Corey Noble which made acquisition of the cages possible.

TRANS TIME MONKEYS AROUND

On April 14, 1981, a Japanese film crew from the Asahi television company recorded the Trans Time suspension team as it placed two monkeys into suspended animation. One monkey was anesthetized, received a thoracotomy and was subjected to a total body washout followed by glycerolization to approximately 3M. This animal was then cooled to dry ice temperature in an alcohol bath, followed by slow cooling to liquid nitrogen temperature. The Japanese wished to document the techniques used for placing human beings into cryostasis.

The second monkey was cooled to 6°C while on a respirator under anesthesia and then was rewarmed to 37°C. While good cardiac recovery was initially seen on this animal, for some reason not yet clear the animal developed arrythmias during the end of the rewarming procedure and suffered irreversible cardiac arrest. Both monkeys were of the Cebus variety and weighed a little under 5 pounds. The animal which is currently in storage in liquid nitrogen will remain so for at least another year, until it can be evaluated for morphological and biological changes via electron and light microscopy and histochemistry.

We congratulate Trans Time on a successful and exciting weekend, and Jerry Leaf as well, who flew up from Los Angeles to head the surgical team for TT. We hope to have a much more detailed report on all aspects of this story in the near future.

BACK FROM THE "DEAD"

The May 1981 issue of READER"S DIGEST carrys a fascinating article entitled "The Baby Who Came Back from the Dead." It documents clinical research now underway at San Diego Children's Hospital and being conducted by Dr. Bradley Peterson of the Pediatric Intensive Care Unit. The article reports on the recovery of an 11-month old baby girl who, after a drowning incident in an apparently heated jacuzzi, was without respiration or heartbeat for 45 minutes. Utilizing a technique pioneered some 5 years ago by Drs Peter Safar and Achiel Belyaert of the University Health Center in Pittsburgh, Peterson is treating victims of prolonged ischemia by placing them in a barbiturate coma. Barbiturate-induced comas reduce the CNS metabolic demand and minimize cerebral edema--both of which are factors implicated in the failure of current medicine to successfully resuscitate the brain after prolonged ischemia.

At a recent FIBER meeting in Washington, D.C., papers were presented by Harry Demopolus on successful cerebral resuscitation of both children and adults after periods of up to one hour of normothermic or near normothermic ischemia. These developments will probably come as no surprise to cryonicists who have anticipated such clinical developments since Hossman and Sato first published their work on recovery of the cat brain from prolonged periods of normothermic ischemia -- almost a decade ago.

As the 4-year old brother of the little girl who recovered from nearly an hour of what is even now considered "biological death" nicely summed up, "Allison fell into the jacuzzi and died. But now she's alive!" As for the ethical questions in all of this, the family, according to READER"S DIGEST, is also simply rejoicing that "now she's alive." We are too!

THIOPENTAL, pH, and ISCHEMIA

Edwin M. Nemoto and Stanley Frinak report in the Jan-Feb issue of STROKE (Vol 12, #1, 1981) on the failure of Thiopental to speed renormalization of cortical pH following ischemic episodes of 15 minutes at normothermia. Barbiturates are known to improve cerebral recovery if administered following an ischemic insult (see the above story), and one mechanism used to explain this is barbiturates ability to decrease cerebral metabolism and raise cerebral pH in the normal brain.

Nemoto and Frink found that after the first 5 minutes of ischemia brain pH dropped to 6.2 on the average and remained unchanged until cerebral circulation was reestablished. Following cerebral recirculation, brain ph did not immediately begin to normalize but rather dropped a little more (probably as a result of increased lactic acidosis as the brain is resaturated with glucose.) At 30 minutes postischemia, brain pH had returned to control values of 7.02 in both groups, and Thiopental seemed to have no effect on renormalization. Apparently, barbiturates' activity in protecting the brain from ischemic injury does not result from preventing acidosis.

LANSING EFFECT FOR PEOPLE TOO?

In 1947 A.I. Lansing (Journal of Gerontology 2, 8228-39) documented that the lifespan of rotifers, small microscopic freshwater animals, decreased steadily with the age at which reproduction in the population was allowed. Thus, if the rotifers were allowed to reproduce only near the end of their

fertile period, the offspring lived shorter lives. Now, it seems a similar effect has been documented in humans with respect to the likelihood of contracting the most common form of senility -- Alzheimer's disease.

Two researchers at University of Washington, Donna Cohen and Carl Eisdorfer, have reported on 80 patients suffering from Alzheimer's disease. All the patients in the study were first born children and the median ages of the patients' mothers and fathers at reproduction were 35.5 and 38 years, respectively. These reproductive ages are 10 years greater than the average for the general population.

This finding represents the first link between parental age at reproduction and Alheimer's disease. Cohen and Eisdorfer also point out that victims of Down's syndrome, a genetic disease also associated with advanced parental age at reproduction, sometimes suffer a type of brain degeneration which is very close to that observed in Alzheimer's disease. The researchers are urging additional work to establish other possible links between the two diseases.

A NEW LOOK AT THROMBOLYTIC AGENTS FOR CRYOSTASIS?

One of the most difficult to overcome complications of an extended postmortem delay in administering perfusion is gross intravascular clotting. In many cases patients are presented for cryostasis, having had no adequate treatment, with a delay of many hours or even days. While prompt cooling has been shown to minimize many undesirable postmortem changes, it is not particularly effective at inhibiting clotting. Even in cases where prompt resuscitation and heparinization have been achieved, inadequate control of acidosis effectively neutralizes the anticoagulent activity of the heparin. It is with extreme interest that we report on an article which appeared in STROKE magazine(Jan-Feb '81).

"Thrombolytic Therapy in Thrombosis" reports on a recent N.I.H. conference which addressed the clinical treatment of deep vein thrombosis and pulmonary embolism. Conclusions of interest to cryonicists are:

---Thrombolytic therapy employing streptokinase or urokinase effectively lyses thrombi in the deep venous system and emboli in the pulmonary circulation; and the thrombi disappear more rapidly than with heparin therapy.

---Thrombolytic therapy results in a greater improvement of the abnormal hemodynamic responses to pulmonary embolization than is observed with heparin therapy. Pulmonary hypertension may be greatly lessened after as little as 1½ hours of thrombolytic therapy. Improvement of pulmonary hypertension peaks after approximately six hours of therapy.

---Thrombolytic therapy may prevent permanent damage to the pulmonary vascular bed by lysing emboli and restoring the pulmonary circulation to normal. In contrast, it has been demonstrated that residual emboli usually persist in patients treated with heparin alone.

The potential of thrombolytic therapy in cryostasis operations seems good. Much additional work will have to be undertaken to determine the sensitivity of thrombolytic agents to pH as wellas their possible impact on damaged cells.

COMING UP

NALOXONE -- In a future issue of CRYONICS a feature article will describe recent advances in treating hypotension and ischemia by the use of the narcotic antagonist and antioxidant Naloxone. Increasing evidence is accumulating which indicates that Naloxone may be an extremely valuable drug in salvaging victims of severe trauma, septic shock and cardiogenic shock following a heart attack.

YOUR SLIDES MAY BE FADING INTO OBLIVION! --recommendations in the next issue.

FREEDOM, MORALITY, AND THE ETHICS COMMITTEE

by Thomas K. Donaldson, PhD.

Many immortalists by now have begun to obtain and use for themselves some of the chemicals already shown to increase lifespan in animals. All immortalists who have done this will know some of the issues which their activity raises in the minds of others. The therapy is unproven, and may even cause harm. The "disease" is not even recognized to be such by the majority of doctors. Furthermore, virtually all the immortalists who take this action do so independently of medical advice. The fact is that significant numbers of immortalists, right now, have had it with doctors as a class. At the same time, we all know that the doctors do have a lot of expertise, and it will be hard to make much progress in the clinical treatment of aging without their cooperation. Realistically we must expect that for quite a long while we're not going to get much cooperation from any doctors beyond the fringe.

A recent news item gives us a good idea, in brief, of why this situation exists. Recently a doctor from the University of California at L.A., Dr. Martin Cline, used some of the newer gene transplant techniques, previously used only on mice, in the attempt to treat two female patients suffering from an inherited blood disease, Beta Thalassemia Major. Victims of this disease generally have an even shorter lifespan than our own, living only to their early 20's. From the news account it seems that Dr. Cline and his coworkers, before the local Ethics Committee had been able to rule on their case, went to Italy and carried out the gene transplants there. The UCLA Ethics Committee, shortly after the departure of the team for foreign parts, ruled that what they proposed to do was immoral without further animal testing. Karen Mercola, one member of the team, in reply to complaints commented that "You have to remember that we applied this technique to patients who otherwise have no hope."

I believe it may be instructive to examine the probable role of ethics committees in such scientific experimentation. First, it is quite simply false that we would have no ethics if there were no ethics committees. In fact, the formation of ethics committees may very well produce much less ethics than before. Why? At the cost of charges of caricature, let's look at how an ethics committee is likely to work in practice.

Sitting on this Committee will be a large number of eminent scientists and commumity figures. They will be asked to spend eons of their time on the review of cases, all coming to them in identical manilla folders. All members of the Ethics Committee will be chiefly concerned to get this dreary committee work over with so that they can go about more important affairs. On the Committee will be A, a scientist who is chiefly concerned with getting back to his lab; B, a "social activist" who is concerned that the highest moral standards, as defined by him, are upheld; C, a university administrator who is chiefly concerned that none of the powerful and moneyed alumni be offended by the experiments proposed; D, who joined the Committee with the fixed ideathat scientific work in genetics posed a threat to the human race from which it must be protected at the cost of arbitrary amounts of other people's misery; and finally E, who volunteered for the Committee from the English Department with the idea

that his participation may give him useful contacts for his further rise in academia. All members of the Committee, ABCDE, will be looking over their shoulders to make sure that no one from the community at large will object to any experiments they have approved. After all, it simply wouldn't do at all for the Ethics Committee to be accused of approving immoral or unethical work.

Now ethics and morality very much do not consist of doing those things which are universally agreed should be done. Ethics and morality are matters of courage in which someone, a doctor or otherwise, decides to carry out some procedure because he or she has decided that it is moral to do so, EVEN THOUGH some people are going to complain. Think of Joseph Lister, for instance.

By now, centered particularly about the Hastings Institute, a large body of scholars has come forward to tell us what is moral and ethical in these circumstances. I have read their reports myself; I am most impressed by the triviality of what they have to say. Most of the ethical and moral cases discussed, if they had been met in all of their detail and personality by any reader of this magazine, could be as adequately discussed by that reader. All intelligent people know that morality and ethics are never matters of black and white and that in particular cases agreement on what to do will happen rarely if at all. What we have here is not a case of morally concerned people trying to thrash out problems, but of people trying to turn Moral Concern into a career.

Of course the standard argument in favor of ethics committees is that their members will be more disinterested than the scientists and the patients themselves. What we really get are not just disinterested people, but people who are UNintersted in the welfare of particular patients or the outcome of particular treatments. Of course, the doctors and their patients may judge wrongly, but the Ethics Commitee is guaranteed to judge wrongly. Moral questions are for those people who are particularly concerned to deal with as best they can. Busybodies have not been invited to the party.

The end effect of ethics committees is to make sure that little morality and less science is actually practiced. They constitute one of the many problems which practicing immortalists must surmount before we can expect much real clinical progress with aging. After all, experiments with animals are all very well, but we must expect an uproar every time these treatments are applied to people!

Once we understand our problem we can also begin to suggest some ways around it. What we must first do, of course, is to stop leaving responsibility for what we take to our doctors. If the clinician is not actually responsible for supply of a drug, then his ethics committee can do nothing. Some of the businesses selling longevity chemicals have already devised partially effective ways around the law. This problem merits a good deal more legal attention. Secondly, we do need the advice of doctors and a register of sensible doctors in particular areas would help longevists a lot. California would probably be the best place to start on this project. If all else fails, interested longevists will simply have to learn about the medicine themselves. Doctors exist who will teach this for a fee, and the books are available in any good library.

SCIENCE REPORTS --- by THOMAS K. DONALDSON, PhD.

Parabiosis, aging and the immune system.

One important method to investigate the changes which take place with aging is to study mice which have been surgically joined together so that their blood and lymph circulates through both animals. The name of such a procedure is parabiosis. Of course, if the animals so joined are sufficiently different genetically (which is exactly what would usually happen), their combined immune systems will simultaneously reject one another and both animals will die. The usual scientific experiments with parabiosis, however, get around this problem by using mice known to coincide on all the immune factors.

The advantage of parabiosis in the study of aging is that it allows us to distinguish experimentally between changes in hormones of aging animals and changes in their cells not caused by hormones. For the hormones and/or other factors circulating in the blood of parabiotic mice will be identical. Some scientists have already used this technique to study aging. (Ludwig, FC; Elashoff, RM; TRANS NY ACAD SCI 134: 1972. 582-7).

A much more recent and quite interesting study has just appeared in which parabiosis was used to attempt to discover reasons for the decline of the immune system in aging mice. G.M. Butzenko and I.B. Gubrii, of the Institute of Gerontology in Kiev, USSR, report in EXPERIMENTAL GERONTOLOGY (15: 1980. 605-10) on a very thorough study of the immune system in parabiotic mice. Two mice of different ages, one 3 months old and the other 22 months old, were parabiotically joined. The immune system of the old animal was little improved by that of the young animal joined to it. On the other hand, the immune response of the younger animal markedly deteriorated, and in the same way it occurs in the old. Butzenko and Gubrii tried several modifications of their experiment. Some researchers had found that removing the spleen of old animals will increase their lifespans (Makinodan et al, J AM GERIATRIC SOC 24: 1976. 249), so Butzenko and Gubrii studied another set of parabiotic animals in which the older animals had had their spleens removed at the time of joining. Unfortunately, this did not improve the immune system of the partners. Again, the authors tried irradiating the old animals to neutralize their immune system. They report that this operation did increase the immune reponse of the pair.

Butzenko and Gubrii feel that their results are good evidence that in the old animals there is some feedback process which is inhibiting their immune system, and that when an old animal is parabiotically joined to a young one, this same process works to inhibit the immune systems of both. They point out an interesting speculation: that with aging, some inhibitory factors normally controlling growth in the young may continue their production and cause deterioration in the old.

Vasectomy and heart attacks.

Vasectomy is by now a very popular operation to control reproduction, particularly among husbands of families which already have a sufficient number of children. On the other hand, one nagging

worry about this operation, increased by reports of experiments on monkeys, is that vasectomy may significantly increase the risk of heart attacks in later life. Since this effect is one which will take many years to reveal itself, it is hard to say anything definite about it. The experiments on animals show that vasectomized rhesus macques will develop sign of atherosclerosis (hardened plaques in the blood vessels) much faster than controls. This effect occurs both when they receive special diets to promote atherosclerosis and when they receive ordinary lab diets. (Clarkson,T.B. et al, J CLIN INVEST 65: 1980. 15-25); (Alexander, N.B. et al, SCIENCE 201: 1978. 538d-541) Whether these results imply anything for human beings remains unknown.

A recent paper by A.M. Walker et al (LANCET 3: Jan 1981) presents some information on this problem for vasectomized human men. In sum, they report that men with vasectomies if anything showed a lower rate of heart disease than those without. Their study was comprised of 4830 men with vasectomies, about 2000 of whom had had vasectomies before 1975, 2307 vasectomized after 1975, and 514 vasectomized before 1971.

Unfortunately this study does not give the ages of the men studied, a very important variable in any study of heart disease, which increases markedly with age. The only indication of this factor is the observation that the population of men studied tended to be younger than the population at large. In view of this factor, the fact that heart attack risk did not increase markedly in the population studied seems weak evidence in favor of the proposition that it will not do so longterm. However, any longevists who have had or are contemplating vasectomies will want to know of this study.

Alcohol and HDL cholesterol.

It is well known that alcohol taken in excess severely damages the health of the user. It seems less well known, even among longevists, that moderate consumption of alcohol may actually increase longevity. An interesting paper on this subject has just appeared in NEW ENGLAND J OF MED (20: 303. 1159). Willet, Hennekens, et al report a study of HDL cholesterol in marathon runners. Increased levels of HDL cholesterol appear to decrease the risk of heart attacks, and marathon runners show such increased levels. However, Willet et al report something more: that when they studied 90 male marathon runners, moderate alcohol consumption seemed to increase HDL cholesterol levels to a degree greater than could be explained merely by the fact that they were marathon runners. Their experiments are not the only work showing such a relation; they cite other earlier work on different populations of men: men at high risk of coronaries (Hulley, S., JAMA 238: 1977. 2269); Italian men (Henze,K. et al, NUTR METABOLISM 21, Suppl 1: 1977. 157) and others.

Many people enjoy a glass of wine with their meals. Willet et al do not feel that their results in themselves justify us consuming wine. Still, longevists should know that increasing our lifespan may not necessarily mean leading an abstemious life free of all pleasures, even quiet ones.

Carbidopa and levodopa.

One very significant drug which may increase longevity is the drug levodopa, or L-dopa. Cotzias et al , in fact, have reported increases in lifespan of as much as 50% in mice which receive large doses of L-dopa throughout their lifespans. (SCIENCE 20: April 1977. 549.) By now some immortalists have already begun to take small doses of L-dopa as one of their longevity drugs (cf Mann, Secrets of Life Extension). However, one barrier to taking higher doses has been the problem of the side effects of L-dopa, which can be quite serious. The most serious side effect is heart arrhythmias, which in susceptible people may lead to sudden heart stoppage and deanimation.

A recent article in ARCHIVES OF NEUROLOGY (37:1980. 723) by W.W. Tourtellotte et al, gives some hope of a way around this problem. The effects of L-dopa consist of its effects on the brain and its effects on the rest of the body, including the heart and stomach. In our bodies there are enzymes which transform L-dopa into the active substance, dopamine. It is dopamine which causes both the positive effects of the drug in the brain and the negative effects on the heart.

One method to help this problem has been to take another chemical, carbidopa, with doses of L-dopa. Carbidopa inhibits the enzymes in the body which turn L-dopa into dopamine, yet does not change its action on the brain. The effect of using carbidopa is to cut down a good deal both on the doses of L-dopa which are required and on the side effects of L-dopa on the heart and stomach (nausea is one of the less serious side effects.) One preparation already marketed under the name Sinemet is exactly such a combination drug and is known to reduce side effects considerably. The recent paper by Tourtellotte et al reports that an even larger dose of carbidopa than that in the present preparation will have improved effects. Their experimental drug contained twice the usual dose of carbidopa and the same amount of L-dopa.

These results are interesting for immortalists in two ways. First, the side effects of L-dopa have inhibited some immortalists from taking it, and means to deal with those side effects are therefore very significant. Secondly, L-dopa itself is very expensive, particularly in the large doses which would be necessary to reproduce, in humans, the effects Cotzias obtained in mice. Any means which allows a smaller dose of L-dopa would therefore have considerable interest.

A metabolic defect in obesity.

Obesity is a serious problem for someone who wants to live a relatively extended lifespan. Even though it appears to be merely a problem of bad habits, the very difficulty obese people have in reducing their food intake would lead any serious thinker to guess that much more fundamental metabolic faults may be at work. Our appetite for food, like all our other physiological characteristics, is regulated by chemical factors in our brain and body, and obese people may suffer from an uncontrolled appetite for food not because of any simple"bad habits," but because their mechanisms for regulating appetite are deranged.

Nevertheless, any such derangement appears to be quite subtle. Animal studies have shown that metabolic defects of this sort can indeed produce obesity (e.g. Chlouverakis, C.; EXPERIENTIA 26: 1970. 1262). However, as yet human evidence has been equivical.

A recent paper in NEW ENGLAND JOURNAL OF MEDICINE (303-18 : 1980. 1017-1022) by M. DeLuise et al, presents some quite specific evidence that one particular metabolic defect may underlie obesity in human beings. DeLuise et al report experiments aimed at finding signs that, in the obese, the metabolic processes which maintain the levels of sodium and potassium inside the body cells are impaired.

As readers know, all body cells are bags of fluid, of a chemical composition differing from that of the fluid outside them. This composition gradient is maintained continuously by metabolic activity of the cell. One important activity of the cell membranes of this sort, the sodium-potassium pump, keeps the concentrations of sodium and potassium different from those of the extracellular fluid, since if left to themselves these substances would diffuse out of the cell membranes and the concentrations would equalize. It has been estimated, in fact, that from 20 to 50 percent of the energy used by our cells is expended in running the sodium pump. This fact connects the activity of the sodium pump to much more visible characteristics of our body. Since the energy expended by the sodium pump will ultimately appear as heat, it follows that the sodium pump must play a considerable role in our ability to maintain a normal body temperature.

DeLuise et al report the results of a study of the sodium pump in the red blood cells taken from 21 obese human subjects compared to those taken from control, non-obese, people. They measured the number of units of the sodium pump active in the membranes of the red blood cells, and found that this number was reduced by 25% in obese subjects as compared to controls. They also found an increased concentration of sodium in the red blood cells of their obese subjects. Even more importantly, they could correlate the percent of reduction in number of sodium pump units in each subject with the degree to which their body weight exceeded their ideal body weight.

These experiments constitute one of the few successful attempts to find signs of a metabolic defect in obese humans. Among its implications is that obese people should also show a defect in their ability to produce and maintain normal body temperature. Unfortunately, these findings don't tell us how to cure the problem; but of course, before we can cure a problem we must first discover the reasons for it.

THE COORDINATED SUSPENDED ANIMATION RESEARCH PROJECT

I. Pilot Study in Total Body Washout

by Michael Darwin, Jerry D. Leaf, and Corey Noble, PhD.

INTRODUCTION

Due to a wide variety of social, political and logistic reasons, the research required to develop a workable technique of suspended animation for mammals is not being undertaken. Recent years have seen a progressive erosion of programs for long term organ preservation via freezing or extreme temperature reduction. Some organ preservationists have suggested that physical and biological problems involved in freezing and thawing large masses of highly differentiated tissue are insolvable, while others have minimized the need for extended preservation on the basis of recent advances in immunology and tissue typing. At least one prominent cryobiologist has specifically indicated opposition to further organ preservation research on the grounds that it might yield whole body human suspended animation as a fallout effect. The cryobiologist viewed this possibility as highly undesirable from a number of social and moral standpoints.

Lack of organization, absence of motivation, poor prospects for funding, pessimism, and in some cases deep moral opposition all clearly preclude any reasonable level of effort being applied by the traditional cryobiologist to develop techniques of mammalian suspended animation any time in the forseeable future.

The Institute for Advanced Biological Studies is not only deeply committed to a program of cryogenic rescue for contemporary people who die, but is greatly concerned that workable techniquesof freezing and rescue be developed to improve the chances of those now living. It is apparemt that widespread application of the cryonics program has not occurred, contrary to early expectations. Despite the fact that many different marketing and public relations approaches have been used, only a minscule portion of the people approached with the idea have become involved on any level. Negligible or very slow growth of the cryonics idea seems likely under the present circumstances.

The factor which appears most responsible for this discouraging picture is the absence of any substantial evidence that the mammalian central nervous system can withstand cooling to and rewarming from very low temperatures. Related to the lack of confidence about the efficacy of the procedure itself is the associated pyschological problem of the procedure being undertaken only after a declaration of legal death has been made. The advent of workable suspension techniques for the central nervous system would very likely eliminate the necessity of applying the treatment after legal death has occurred.

In order to overcome these problems, the Institute for Advanced Biological Studies and the Institute for Cryonic Education, two nonprofit, tax-exempt research organizations, as well as Soma Incorporated, a profit-oriented cryonics company have banded together to undertake a coordinated program of research whose long term goal is successful cryopreservation of the mammalian brain. The goal of the initial experiment, reported on the following pages, was to gain experience with the reversible induction of deep hypothermia and total body washout in the canine model and to evaluate the suitability of a proposed perfusate for maintaining the brain. The experiment took place at the IABS Laboratory in Indianapolis, Indiana on August 21, 1979.

MATERIALS AND METHODS

Experimental Animals: Two large dogs were employed as the experimental subjects. One animal, weighing 30 kg. and of Husky mix, was sacrificed and 5 units of citrate-phosphate-dextrose preserved blood was collected for use in priming the oxygenator and supporting the experimental animal during the post-operative period. The experimental animal was a 25 kg. black and brown male of Doberman mix.

The pump and oxygenator prime consisted of 500cc normal saline, 1 gram ascorbic acid, 75cc o.3 M Tham, 250cc Plasmanate (serum albumin 5%), 75cc Dextran 70 (36%) with 10% glucose in normal saline, and 1,500cc CPD banked blood.

The oxygenator employed was a Bently BOS-10 with Tygon S-50-HL tubing used as conductive line. Dow-Corning medical grade silastic tubing (½" I.D.) was used for the pump shoes. A 40-micron Pall filter (EC3840) was used to guard the arterial line. A Sarns torpedo heat exchanger was used for cooling and rewarming.

Pre-operative medication was 45mg Thorazine administered intramuscularly. General anesthesia was secured with Diabutal administered intravenously at 33 mg/kg. Shivering was prevented during the procedure by the administration of 3 mg. Metubine initially and as needed.

Surface cooling was achieved by immersing the animal in a tub of crushed ice and water. Respiration was supported on a Bennett PR-1 resirator and the EKG was continuously monitored. In order to achieve slight hemodilution and maintain a patent I.V., 250cc of normal saline was administered during the surface cooling to 28°C.

Partial bypass was achieved via the juglar, femoral, juglar approach. Venous return consisted of USCI 18 Fr. veno/arterial cannula anchored in the right external jugualr vein and right femoral vein. Arterial perfusion was retrograde through the right femoral artery employing a custom glass cannula approximately 8 Fr. in size. All distal vessels were snared closed with umbilical tape and 3" sections of Red Robinson catheter. Cannulas were also secured with umbilical tape and Red Robinson snares.

Partial bypass was initiated without incident. Arterial pressure was monitored through a 17 gauge angiocath introduced through a 3/8" connector Luer port of the ½ x ½ x ½ Bently Y-connector and advanced beyond the end of the 18 Fr. venous cannula in the external jugualr and into the right atrium. Pump cooling proceeded smoothly and without incident. Baseline arterial pressure at the start of extracorporeal circulation was 100 mm Hg and the CVP was 4 mm Hg. Pump output was adjusted to 1 liter per minute for cooling to 10°C. 100% oxygen was administered at a flow rate of 6 liters er minute to reduce CO_2 and increase pH. Tham was administered below 22°C in order to raise the pH to an appropriate level. A final pH of 8.0 was achieved. A final temperature of 13°C was reached before arresting perfusion prior to attempting total body washout. The animals blood was allowed to drain into the oxygenator during the period of circulatory arrest.

The perfusate was a newly-developed high glucose, moderate potassium solution containing glutathione as a reducing agent and aenine to minimize depletion of intracelluiar high energy reserves. It was initially decided to use 70,000 molecular weight Dextran in the form of Hyskon hysteroscopy fluid as the sole colloid. Unfortunately, due to a defective osmomometer, the colloid osmotic pressure of the Hyskon solution was determined to be incorrectly low. Consequently, the Hyskon was supplemented by the addition of 178,000 molecular weight Dextran of "clinical grade" supplied by Sigma Chemical Company of St. Louis, Missouri. Great difficulty was encountered in dissolving this material

(which is characteristic of Dextran which is not adequately prepared). Due to the high viscosity and/or the presence of particulates related to the presence of the 178,000 MW Dextran, only about 600 to 800cc of the perfusate could be forced through the 0.2 micron sterilizing filter and into the blood circuit. Attempts at prefiltration with a 40 micron Bently blood filter were unsuccessful in preventing immediate development of high resisitance in the 0.2 micron filter.

RESULTS AND DISCUSSION

External cooling to 28°C proceeded very smoothly at a rate of approximately 1°C per minute. It is apparent that, with adequate surface refrigeration, external cooling in the absence of shivering is very effective in an animal this size. The heart rate of the animal throughout most of the external cool-down to 28°C was between 142 at the start of the procedure to 72 during the period of hypothermic autoperfusion at 26°C immediately prior to initiation of partial bypass. Respiration was adjusted from 14/min at 37°C to 12/min at 28°C.

At the time total body washout was attempted, the dog had been in suspended animation at 16°C for 32 minutes. At this time the condition of the animal was considered to be excellent; initial cooling had gone smoothly; tissue perfusion and oxygenation was judged to be good; and no major crises had developed. There appeared to be every indication that the experiment would be successfully concluded. Once it became apparent that the total body washout was not possible owing to the difficulties with the perfusate, it was decided to attempt rewarming and resuscitation of the animal.

Rewarming proceeded smoothly at a rate of 0.6 degree per minute. A Travenol RSP kidney machine was employed as a source of warm wall water for rewarming to 37°C.

Soon after rewarming was begun, it was noted that the animal had begun to bleed profusely from the sinuses. Almost immediately after this development was noted, the CVP began to rise to a high of 20mm Hg. Arterial and venous pH readings were within physiological limits. Surprisingly, the heart did not resume beating spontaneously upon rewarming to 30°C. External massage changed a flat EKG to ventricular tachycardia. Electrical defibrillation was finally successful after repeated efforts, including the administration of sodium bicarbonate, adrenaline and calcium chloride.

After resumption of sinus rhythm the arterial pressure remained low: 40 to 50 mm Hg. Despite the fact that the pump output was set at 1400cc/min, which should have been adequate for partial bypass during hypothermia, the heart did not recover. Attempts to wean the animal from partial bypass resulted in a rapidly rising CVP and declining arterial pressure.

Pulmonary resistance due to thrombosis induced by the Dextran 180 could account for both venous congestion and reduced cardiac output due to low left atrial and low left ventricular volume. The increased heart rate that was observed could not compensate for the degree of low ventricular filling. The low arterial pressure led to a further reduction in perfusion pressure, due to inadequate myocardial perfusion and eventually to complete cardiac failure. A peripheral vasoconstrictor such as a neosynephrine could have increased the arterial pressure on partial bypass, but the pulmonary insufficiency would have made it impossible to discontinue pump support. Pulmonary edema would have determined the final outcome, as it did. Pulmonary blood in the respirator ended the experiment.

RETROSPECTIVE

Further investigation of the characteristics of the Dextran 178 supplied by Sigma revealed that the material was only partially hydrolyzed and the designation "clinical grade" implied only that it was pyrogen-free. Previous investigations have indicated that unhydrolyzed or partially hydrolyzed Dextrans are extremely injurious to parenchymatous organs and actually cause sludging and coagulation problems -- the exact opposite of the desirable characteristics exhibited by the lower molecular weight Dextrans. In the future, high molecular weight Dextrans and unhydrolyzed Dextrans should be avoided in both clinical and experimental situations.

Subsequent evaluation of the viscosity of Dextran 70-containing perfusates indicate that 6% soluations can be pumped at a flow rate of 4 liters/minute at 3°C through a 0.2 micron pall filter (Ultipor DFA 3001 ARA). In the future all perfusates should be tested for viscosity and handling properties prior to experimental application.

Even before the addition of the Dextran 178, serious difficulty was encountered in dissolving the adenine in the perfusate. Since the experiment was undertaken we have evaluated adenine HCL as an alternative to pure, crystalline adenine and found that it dissolves rapidly with no difficulty or extended stirring.

Many logistic problems were encountered, not the least of which was the excessive amount of time consumed by weighing out and mixing perfusate chemicals. More adequate preparation of perfusate components in the future should eliminate this difficulty. Also, several desirable supplies were not on hand due to financial limitations as well as to a lach of experience with this model. In the future a more rigorous checklist approach should be applied, and hopefully more adequate funding will eliminate the absence of urgently-needed equipment, such as a top-loading balance scale and a large capacity stirring machine.

PROSPECTIVE

Having learned from all these points, we are now in a much better position to succeed with our next experiment. We must also conclude that most of the technical problems of inducing deep hypothermia and achieving total body washout were solved by the equipment and techniques we employed, including surface cooling, sterile technique, cardiac monitoring, mechanical ventilation, perfusion, and defibrillation.

The total body washout model has the advantages of 1) allowing behavorial criteria of brain function to be examined after preservation, and 2) providing an optimum environment for cerebral repair after preservation. The degree of success which was experienced in this initial pilot endeavor indicates that necessary preliminary questions, such as the optimal base perfusate for extended hypothermic brain preservation, can be answered in the near future. The sponsoring organizations thank all who participated and invite all interested individuals and organizations to participate in the long term preservation project.

ACKNOWLEDGEMENTS

IABS would like to extend special thanks to Jerry Leaf, whose superhuman effort and commitment were responsible for most that was learned, and to Dr. Noble for his financial and professional support. Also, deep thanks go to Ella Vinci, Brian Cox PhD, Joe Allen, Steve Bridge, Anna Schoppenhorst, Carolyn Doyle, Brian Bock, Allen Lopp, Greg Noe', and to any others we may have omitted.

INSTITUTE FOR ADVANCED
BIOLOGICAL STUDIES, INC.
2901 N. Pennsylvania St. Indianapolis, IN 46205

TO: