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BAY AREA CRYONICS SOCIETY BEGINS NEWSLETTER!

BACS has decided to make available to its membership (on a strictly experimental basis) a copy of the BACS NOTEBOOK, which it plans to send out along with the BACS minutes following each bi-monthly BACS meeting. The BACS NOTEBOOK will be published on a trial basis, and will consist of scientific, business and other kinds of items which we hope will interest our readership. Trans Time has agreed to provide duplicating, clerical, and editorial assistance. The BACS Secretary will assist in gathering and collating information, and other BACS Governors have pledged to provide articles for enclosure. BACS hopes that this notebook will succeed in conveying to its membership a more accurate description of what we are all about; a task which has somehow eluded other cryonics publications. Here's hoping it works.

Paul Segall
June 7, 1984

Life Extension Sciences Research Program

Progress Report - May 31, 1984 sent by BioPhysical Research and Development, a BACS research contractor, to Saul Kent of the Life Extension Foundation of Hollywood, Florida

We would like to report on the research sponsored by the Life Extension Foundation. In January, 1984, BioPhysical Research and Development (BPRD) was awarded a $5000.00 grant to investigate the reversible total body washout of hypothermic hamsters, and to study the possibility of these animals surviving liquid-solid state conversions. During the period financed by the award, we developed several techniques for machine-controlled artificial respiration and blood substitute perfusion, including the construction of a ventilation apparatus to serve as a duplicate respiratory device when the Harvard Apparatus obtained on loan from the University of California was being employed in other experiments. Arterial cannula design was improved; the outside diameter being reduced while retaining the maximum inside diameter for maximum fluid flow. The technique of venous cannulation was upgraded as well, both arterial and venous cannulae now end in luer fittings permitting connection to transducers and 3-way stopcocks, and ultimately allowing fluid access to the circulatory system. They also are equipped with sharpened metal inserts to aid their insertion into the vasculature. An apparatus was constructed for the manufacture of the new cannulas. Duplicate laboratories were equipped for this project, both in the Department of Physiology-Anatomy at the University of

California at Berkeley, and at BPRD's own in-house workshop.

More than 35 individual experiments were conducted involving the perfusion of hypothermic hamsters. The investigators became more familiar with the complicated surgical techniques involved, and some important correlates of survival following this arduous and unusual procedure were discovered. It was noted that the state of the heart was useful in predicting which hamsters would survive. Hamsters whose hearts had stopped were less likely to recover than those which continued to beat steadily near the ice-point. If the pressure produced by the roller pump perfusing the circulation was diminished at low temperature, the heart was observed to slow considerably, and often would soon stop. Even if the pressure was then raised, the probability of recovery was little. However, in one case, after re-perfusion with whole blood was completed, the animal revived when the jugular vein was tied off and the carotid artery continued to be perfused for some time, thereby elevating systemic circulatory pressure. These findings are consistent with new concepts of no-reflow as a cause of ischemic brain death, and suggest that once the cerebral vasculature is constricted that considerable pressure may be needed to re-establish circulation.

Current theories cite the accumulation of Co** in the cerebral vasculature as a key factor behind this constriction. Our research thus suggests that the use of low Ca^{++} perfusates, as well as Ca^{++} channel blocking pharmacological© might be useful in preventing the no-reflow response.

In our studies, we have investigated the use of ketamine to replace the closed jar technique. We have been able to revive two ketamine-anaesthetized hamsters following total body washout near the ice-point. These hamsters were perfused with blood collected from blood donors which also were anaesthetized with ketamine. Thus, we have established the possibility of using this pharmaceutical to prepare for the induction of hypothermia by whole body immersion in crushed ice. This may have some advantage in avoiding possible injury due to excessive hypoxia and hypercapnia experienced in the closed jar technique. It is also more convenient, an important factor in such a complex and lengthy protocol.

New techniques of blood collection were pioneered, whereby heparin was injected into the heart of the blood-donor hamster to prevent clotting and therefore allowing the harvesting of more blood. Additionally, we have used blood collected during the early stages of blood substitute perfusion (the first pass) when commencing blood re-infusion. This reduces the number of hamsters required for sacrifice, as well as shortening preparation time. These time-shortening advances allow the use of fresh blood during each perfusion, eliminating the complications of blood storage from many experiments.

The above research was documented on videotape and presented at the Lake Tahoe Life Extension Festival held on Sunday, May 27, 1984. An abstract including this research was also prepared and submitted for presentation at the upcoming Cryo 84 Conference sponsored by the Cryobiology Society and scheduled for San Diego later this summer.

In one experiment, a hamster was perfused with an 0.3M solution of glycerol, a concentration similar to that found in the body fluids of frogs surviving partial freezing overwinter. In this hamster the heart continued to beat slowly during the 5 minutes that this glycerol-containing perfusate was administered; the animal's body temperature reading approximately 3°C. The perfusion was stopped for an additional 5 minutes, and the hamster was allowed to rest with the perfusate remaining in its vasculature. The animal was re-infused with declining concentrations of glycerol, and then perfused with blood. Although the animal never recovered (presumably due to surgical error), the heart rate did increase to as much as 65 beats per minute, suggesting that the glycerol was not immediately toxic to the perfused hamster.

The above studies are being continued under grants made available to BPRD from the Foundation For the Enhancement and Extension of Life through the Bay Area Cryonics Society,Inc. We wish to thank the directors of the Life Extension Foundation for their generosity, and we hope that further collaboration in the field of life extension research will be possible in the near future.

Progress Report - June 7, 1984 sent by BACS to The Foundation For the Enhancement and Extension of Life describing work performed by its research contractor BioPhysical Research and Development.

The major area of concentration has been on the Hamster Suspension Project. However, the House Cloning Project, as well as the Rodent Anti-Aging Project have also received some attention. Additionally, there has been some effort to acquire more lab space, which will be necessary to any expanded version of the program.

Recent work on the Hamster Suspension Project has centered around the development of instrumentation. Dr. Paul Segall and University of California Senior Sandra Gan have been able to surgically interface the circulatory system of hamsters with three-way stopcocks, allowing for transducer measurements of both arterial and venous blood pressure during the induction of and the revival from deep hypothermia (although calibration and precise quantitation of these variables are still under study). Furthermore, the installation of the stopcocks permit the withdrawal of samples of both perfusate and venous effluent at any time during the procedure, thereby permitting determination

of pH, chemical constituents, blood gases, hemoglobin concentration, hematocrit and other information as needed. Thermistor leads have also been adapted on-line to a recording polygraph, providing a continuous record of deep body temperature.

Several new surgical variations have been introduced, including an innovation developed by Pacific Medical Center biochemist Dr. Hal Sternberg, who has been donating his Saturdays to the mastery of the intricate microsurgery involved in these perfusions. We have recently added the use of a screen to suspend the hamster over a well filled with ice which permits warm-up by simply pouring warm water over the animal to melt the ice, the water being then evacuated through a drain at the bottom of the well. This technique takes more complete advantage of the simple but ingenious design of Dr. Harold Waitz's operating stage, thus allowing for large temperature changes without the necessity of moving the hamster being operated on. The importance of this can not be over-stressed, as moving animals while instrumented and intubated microsurgically has rarely been done without extreme risk to the entire experiment.

Dr. Waitz has been regenerating a blood gas apparatus obtained on loan from Trans Time, which will provide information on the CO_2, O_2 and pH of minute samples of blood. He has obtained some calcium blockers and is setting up to investigate their effectiveness in supporting viability in hamsters subjected to extended periods of hypothermic anoxia. He is also exploring the use of a micro-computerized accounting system to handle record-keeping of financial support.

Our immediate goals for next month include the induction of and revival from deep hypothermic states while monitoring changes in blood pressure, EKG, deep body temperature, pH, hematocrit, and, if possible, blood gases. We will then compare these measurements to similar ones made before and after total body washout, in order to determine factors contributing to revival. A television crew from Los Angeles has made an appointment to videotape our experiments, as well as to interview us for a PBS documentary on life extension. We are hoping to be able to successfully demonstrate our new techniques for them when they arrive at the end of July. We also have to begin preparing for our presentation at the Cryobiology conference scheduled for San Diego at the end of August, provided of course that our submitted abstract has been accepted. (We were told we would hear soon as to its status.)

Greg Cole, a Berkeley doctoral student, and Segall have been discussing a new attack on aging based on a recently appearing article (copy enclosed) by UCLA's Dr. Roy Walford and his associates. The article describes the retention of a very specific form of eye lens protein in underfed juvenile mice,

while normal mice lose the ability to produce this protein early in the course of aging. When Cole brought this article to his attention, Segall was reminded of the long-term retention of egg cells by immature rats when fed his low tryptophan diets. He hypothesized that early and severe nutritional restriction in rodents may delay aging by preventing loss of very specific cell types. Aging may therefore occur due to a programmed cascade of specific cell loss, in which certain maturation-related phenomena (for instance, rising steroid levels), cause the loss of specific groups of cells, which thus disappear and possibly deprive the organism of their products and/or functions. The loss of these cell products and/or functions thus engender the loss of other cell types dependent on the first set of cells. The loss of one kind of cell promotes the loss of a subsequent group, eventually creating the too-familiar scenario of senescence. This theory, if correct, allows for the discovery of these specific cells and the particular order in which they are lost, as monoclonal antibodies can be made to each protein which disappears. These antibodies can be tagged (by radiolabel, fluorescent or heavy metal dye), and followed back to the original kind of cell where the lost protein originated from.

The above techniques, and others, would then allow us to develop a "schedule" of precisely which cells are lost at exactly which times during and after maturation, and a detailed mapping of the course of aging would result. In the rat or mouse, animals which age in about 2 years, the entire aging process could be chronicled. This would allow for a detailed understanding of aging; the principles learned, of course, could then be applied to higher mammals.

The Foundation For Infinite Survival, a Berkeley-based life extension organization, has donated several mice of the C57BL strain which is commonly used in aging research. We have begun a mouse colony at the BioPhysical Research and Development laboratory which, after two litters, has now grown to nearly 25 animals. Plans are underway to upgrade our animal storage shed by adding air conditioning and a controlled environment mouse colony isolation unit (hopefully fashioned out of a discarded glass door delicatessen display refrigerator, which we are currently searching for). An abstract based on our new view of aging (copy enclosed) has been sent to the American Aging Association for presentation at its upcoming October, 1984 meeting in New York City. It is hoped that discussion of these ideas within the gerontology community will confirm or improve the hypothesis.

Along with the study of aging, the mouse colony will be used as source of animals for the Mouse Cloning Project. Drs. Segall, Waitz and Weintraub, (a molecular biologist with extensive tissue culture experience) visited the Laboratory of Radiobiology at the University of California at San Francisco

Medical Center, where Dr. Roger Pederson and his associate Dr. Akiko Spindle described current techniques for the isolation and handling of fertilized mouse eggs. Dr. Susan Weintraub, formerly of Cutter Laboratories, the University of California at Berkeley and Cornell University Medical Center, is joining us in this project in which she will develop in-house techniques for nuclear transplantation and surrogate motherhood in the mouse. Segall has applied for admission to the 1984 Gordon Conference on embryogenesis and development co-chaired by Dr. Pederson planned for the third week of August in New Hampshire. Although coming immediately before the Cryobiology conference, and therefore somewhat inconvenient, this conference will bring together many experts in the areas of mammalian embryology and nuclear transplantation.

That it is especially important to keep in close contact with others in this fast-changing field is exemplified by the following story. Word is circulating around the nuclear transplantation community that the creation of the homozygous diploid, a technique once believed promising for the production of rejection-free organs for transplantation and reported by Peter Hoppe and Karl Illmensee in the Proceedings of the National Academy of Science, has been impossible to repeat. Some have even implied that the claims made about this work may be unreliable. As this technique and its implications are important to us, it is critical for us to get more information about this and related studies. Many of the people active in nuclear transplantation biology are expected to attend this conference, and therefore, if present, we may be in a position to learn something of relevance to this matter.

As laboratory space is already becoming a severe limitation, we have begun negotiations with Trans Time, Inc, and its associates, for the acquisition of more room to work. Trans Time is intensively looking for a new facility as it must vacate its present premises by the end of September, 1984. A building fund dedicated to financing such a facility has raised nearly $25,000, and other Trans Time affiliates are discussing additional investment. Several properties have been located, including a vacant mortuary with 4-5000 square feet of usable space. If Trans Time buys the building, located in neighboring Oakland, California, we could rent up to 2000 square feet of laboratory space for $1000-^1500 per month. This would be especially beneficial when our studies reach an expanded stage.

Basically, the above is a reasonable summary of where we stand now. We thank the Foundation For the Enhancement and Extension of Life for its continued support, and look forward to more opportunities to report progress in the near future.

BACS-Sponsored Research Accepted for Cryo 84

The following abstract was accepted for presentation at the Cryobiology Society's up-coming Cryo 84 conference scheduled for the campus of the University of California at San Diego, beginning August 21, 1984. The abstract describes research sponsored by the Life Extension Foundation and BACS, performed by BPRD at its own in-house facility and in the Department of Physiology-Anatomy at the University of California at Berkeley.

REVIVING HAMSTERS AFTER HYPOTHERMIC ASANGUINOUS PERFUSION

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A technique has been developed allowing the revival of Syrian golden hamsters, Mesocricetus auratus. following total body washout at temperatures within a few degrees of the ice-point. Five hamsters were place into hypothermic states (4 using the closed jar technique of Smith and Andjus, 1 by injection with ketamine), and then subsequently packed in crushed ice. After body temperatures fell below 13°C, the animals were removed from the ice and placed ventral side up on ice-filled plastic bags resting in the field of a stereo-microscope. Rectal temperatures and EKG were continually monitored, and artificial respiration was administered.

Incisions were made to the right of the midline of the neck immediately above the clavicle and both the jugular vein and the carotid arteries were microsurgically exposed. The temperature was lowered to 8-5°C and a specially prepared heat drawn fluorocarbon cannula was inserted into the carotid artery. An ice-chilled blood substitute consisting of 6% Dextran 40 in Ringers Lactate buffered to pH 8.4 was pumped into the arterial system at a rate of 0.5-1.0 ml./min. After a few seconds, the jugular vein was cannulated and the venous effluent collected. Perfusion continued at this rate until 4-5 passes were completed and the hematocrit fell below 2.

After time periods varying from 5-30 minutes, body temperatures declined to 1-3°C. The hamsters were then perfused with a mixture consisting of 50% hamster blood and 50% blood substitute, re-warmed slightly while being artificially respired, and re-perfused with whole blood. Re-warming and artificial respiration were continued until voluntary respiration and movement were observed. The cannulas were removed and the surgical wounds were closed. Animals survived 1-6 hours after regaining consciousness; hemorrhagic shock due

to anti-coagulation and surgical trauma are suspected in the eventual cause of death.

New Theory of Aging Offered

Below is an abstract describing a new theory of aging submitted for presentation at the upcoming meeting of the American Aging Association (AGE), scheduled for the Roosevelt Hotel, 45th Street and Madison Avenue in New York City beginning October 18, 1984 in New York City:

AGING AS A PROGRAMMED CASCADE OF SPECIFIC CELL DEATH. Paul E. Segal1. Dept. Physiology-Anatomy, U. of Calif. at Berkeley, Berkeley, CA 94720

It is hypothesized that dietary restriction delays aging by slowing specific cell loss.

Severe early nutritional restriction retards cell loss and proliferation. Restricted diets have recently been shown by Leveille et. al. to delay loss of specific proteins in mouse eye lens. These proteins are produced during the elongation of lens fiber cells after proliferation from the equatorial epithelial monolayer. During the first year of adult life in mice, these gamma crystallin lens proteins disappear in normal mice but are retained in those subject to early dietary restriction. By slowing cell proliferation, the restricted diets may also slow compaction into the lens center, and concurrent loss of cellular protein synthetic capabilities.

Severe tryptophan deficient diets, when initiated in the juvenile rat, delayed loss of ova. Neurotransmitter and hormonal declines in older rats suggest that there may be loss of certain brain and endocrine cells. As low tryptophan diets can slow some of these neurotransmitter and hormone alterations, rats subjected to these diets, as well as caloric restriction, should be examined for specific cell loss compared to age-matched controls.

Trans Time Looking For Investors

Trans Time, Inc. has begun a search for large scale investors by establishing contact with Schuyler Williams, an attorney with the prestigious Pillsbury, Madison and Sutro. This well-known San Francisco law firm was instrumental in developing investment capital for other biotech firms such as Cetus and Genentech. Attorney Williams met with Trans Time directors at their facility last month, and outlined procedures which her firm would use to locate investment capital.

New Promotional Material Designed By BACS

The promotional material appearing on the following two pages was designed by BACS to broaden its base of public support.

BAY AREA CRYONICS SOCIETY, INC.
1098 Euclid Avenue, Berkeley, CA 94708

What Most People Haven't Heard About

CRYONICS

» Preserving Endangered Species

* Protecting Patients Needing Transplants

* Whole Body Storage of Organ Donors

« Emergency Space Medicine

« Delaying the Aging Process

« Opening New Frontiers In Surgery

Most of us are familiar with the Cryonics Society's efforts to provide suspension facilities, techniques and arrangements for people choosing this alternative. However, what has escaped the attention of many are the following lesser known but important Cryonics Society goals.

Cryonics Society sponsored research laboratories are probably the only scientific institutions now attempting to place intact laboratory mammals in a state of reversible, whole body suspended animation. Our researchers are leaders in the field of experimental hypothermic perfusion, and have successfully conducted experiments in which chilled laboratory animals have been revived following the total replacement of their entire blood volume with blood substitutes. These blood substitutes are being studied for their value in protecting tissues against the damaging effects of cold.

The value of these experiments to the preservation of endangered species is obvious, but only Cryonics sponsored laboratories study these techniques. Scientists of the Cryonics Societies have a strong commitment to the study of nuclear transplantation and cloning; research options which may eventually allow us to produce thousands of copies of a near-extinct species using the preserved cells from only one member.

The media keeps us all aware of the many young children, as well as adults, who die because organs for transplant are not

available in time. The Cryonics Societies are the only organization sponsoring research into long term whole body preservation after death, research with particular application to this pressing national problem.

Cryonics researchers, seeking to develop short-term suspension techniques to be used by people facing immediate medical problems, are investigating the partially frozen states employed by overwintering animals such as frogs and insects. They are exploring ways to save people needing transplants immediately who cannot survive the days or weeks often necessary to obtain compatible grafts.

Short-term partially frozen states, achieved temporarily in laboratory mammals by scientists as long as twenty years ago, are now being studied using modern cryobiological approaches, and are under consideration by Cryonics researchers for their potential usefulness in space medicine. Astronauts, often males in their late thirties and forties, and despite rigorous training and careful selection, are susceptible to rapidly progressive pathology such as cancer and heart disease while on lengthy space flight missions. The development of techniques which would allow their temporary suspension for a few weeks or months, until they could be returned to terrestrial medical centers for proper treatment, could potentially save thousands of lives in the coming space age, as well as hundreds of millions of dollars in the cost of aborted missions.

Cryonics scientists have made notable contributions in studies of delayed aging. Interventive Gerontology is a priority discipline among Cryonics experimenters, because there is little reason to preserve patients who were suffering from advanced old age and severe age-associated illness if methods of age reversal are not developed. Also, most Cryonicists want to stay as young as possible as long as possible.

Many techniques presently under investigation in Cryonics laboratories hold great promise for surgery and critical care medicine. Blood substitution in chilled patients may allow for lengthy operations on vital organs such as the heart or brain, in the complete absence of blood flow. Emergency patients may be saved who otherwise would be beyond medical rescue. These techniques may also be valuable in removing malignancies from parts of the body which are now surgically inaccessible.

The Cryonics Societies are making tommorrow's high Biotech advances today. Invest in the future. Support Cryonics.

All contributions are State and Federal tax exempt.