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Manrise Technical Review. At this time, the most widely recognized means of increasing the probabilities of surviving clinical death involve the induction of solid state hypothermia, a low temperature state in which chemical and biological processes are essentially arrested. Most information published in MTR will be directly relevant to this subject.

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Volume 2 · Number 5
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MANRISE
TECHNICAL
REVIEW

EDITORIAL

A little over one year ago, our subscribers received the first issue of Manrise Technical Review. At that time, we announced that an instruction manual would be released in late 1971. The first portion of the manual was completed and mailed prior to January 1, 1972, and all sections have since been distributed except for those pertaining to Phase II. The first drafts of Phase II sections have been completed and are currently being revised for publication. We anticipate mailing these to all manual owners prior to January 1, 1973.

Our readers may have wondered "who is implementing these instructions?" and "what operational status exists within various cryonics societies?". We have little information concerning this, despite the fact that manuals have been purchased by all major cryonics societies having a known operational capability. In the last issue of Manrise Technical Review, Walter Runkel described the Cryonics Society of Michigan Van[1], and MTR would welcome more contributions of this kind. Only by knowing the specific levels of readiness attained by various organizations can we develop a perspective concerning standards, progress, and the achievement of goals.

Recently, a new cryonics society, "Alcor" (The Alcor Society for Solid State Hypothermia) was announced in the Outlook[2]. MTR will publish, with time, one or more articles on Alcor's state of preparedness and its research-oriented activities. At this time, a brief overview may be of interest to our subscribers.

Alcor was incorporated in February, 1972. As a non-profit, scientific-educational institution, it has qualified for both state and federal tax exemption. All members of Alcor are anatomical donors, with full provisions for cryonic suspension. An Advisory Board has been appointed including insurance consultants, individuals active in gerontology, and representatives of other cryonics societies.

Members of Alcor's rescue team carry radio paging devices which permit them to be instantly contacted at any location in the 3000 square mile Los Angeles basin. All donors wear special bracelets (*not* Medic Alert) which key all incoming calls directly to rescue team members. Contractual arrangements exist with several morticians, so that adequate facilities are available even if multiple fatalities should occur at the same time. Manrise Corporation is contractually committed to provide perfusion equipment, chemicals, and all required accessory instrumentation in the event of an Alcor emergency.

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THE MECHANISMS OF CRYOINJURY AND CRYOPROTECTION IN MAMMALIAN CELLS: A REVIEW OF THE LITERATURE

by Linda L. Chamberlain

President,
The Alcor Society for Solid State Hypothermia

An understanding of cryoinjury and cryopreservation with protective additives are interdependent. Theories concerning the former are the very basis of an understanding of the latter. Basically, protective additives alter the course of freezing so as to bring the deleterious net effects of vapor pressure differential, dehydration, and solute damage within tolerable limits. This paper looks at the problem of cryoinjury both from a theoretical view and from the viewpoint of what various types of cryoprotective agents accomplish.

The knowledge that hypothermia affects cellular metabolism is not new. Records of freeze-thaw experience date back as far as 200 years (1,2). In the modern science of cryobiology, the study of improved methods of cryopreservation and the prevention of cryoinjury are interdependent and inseparable.

The effective use of hypothermia to preserve biological matter was greatly facilitated in 1949 when Polge, Smith and Parkes (3) discovered the protective qualities of glycerol. Other compounds were tested, but glycerol remained the most effective of additives until the protection of dimethyl sulfoxide (DMSO) was demonstrated by Lovelock and Bishop (4) in 1959. The question of which cryoprotective agent is most effective has received a great deal of attention in the literature ever since. It has been suggested (5) that combining agents with different protective mechanisms may be promising. Although a definitive answer to this ques-

tion is presently yet to be found, there is no question that most tissues survive the freeze-thaw process better when one or more of these additives are used (4,5,6,7,8,9).

Cryoprotective agents, though they protect the cell to varying degrees, are themselves toxic to the cells at higher temperatures (5,10,11). The Farrant perfusion method (12), elaborated upon by Quaife (13) for use in inducing solid state hypothermia in whole mammals, is one means of dealing with this particular problem. The use of cryoprotective agents today is so much an accepted and integral part of cryopreservation that any study of cellular damage caused by freezing must consider the effects of these agents.

The mechanisms of cryoinjury must be studied first on the minimum organizational unit of matter capable of that process that can be referred to as "life". That unit is the cell (14). It should be stated at the first that there is no "typical" cell (2); one must instead think in terms of a "generalized" cell. Let us consider the structural organization that is common to all cells and which clearly relates to the fundamental activities which sustain life.

BASIC STRUCTURE OF THE CELL

The cell is the fundamental structural and functional unit of all living organisms. The biochemical activity within cells is the basis for life's processes (2). This biochemical activity depends upon molecular movement in an aqueous medium. The structural organization of the cell is the key to this activity. If this structure is destroyed, the cellular function is altered accordingly.

Figure 1 shows a "generalized" cell. Water makes up approximately 90% of cell weight (14), and is contained within a covering, or membrane. This covering is sometimes called the *plasma membrane*, and it preserves the form and individuality of the cell. The plasma membrane, which consists of two extremely thin membranes separated by an intervening gap, should not be thought of as "outside" the cell. It is an integral part of the cell as its properties are central to vital biochemical functions.

The plasma membrane helps regulate the internal environment of the cell and protects against invading microrganisms through its characteristic semipermeability. The cell membrane is generally permeable to water and various materials dissolved in water, but under most conditions it is impermeable to colloids (14). The actual degree of permeability to different substances varies markedly and is dependent on conditions both inside and outside the cell such as sugar content, pH, electrical properties, temperature and many other factors. The plasma membrane is

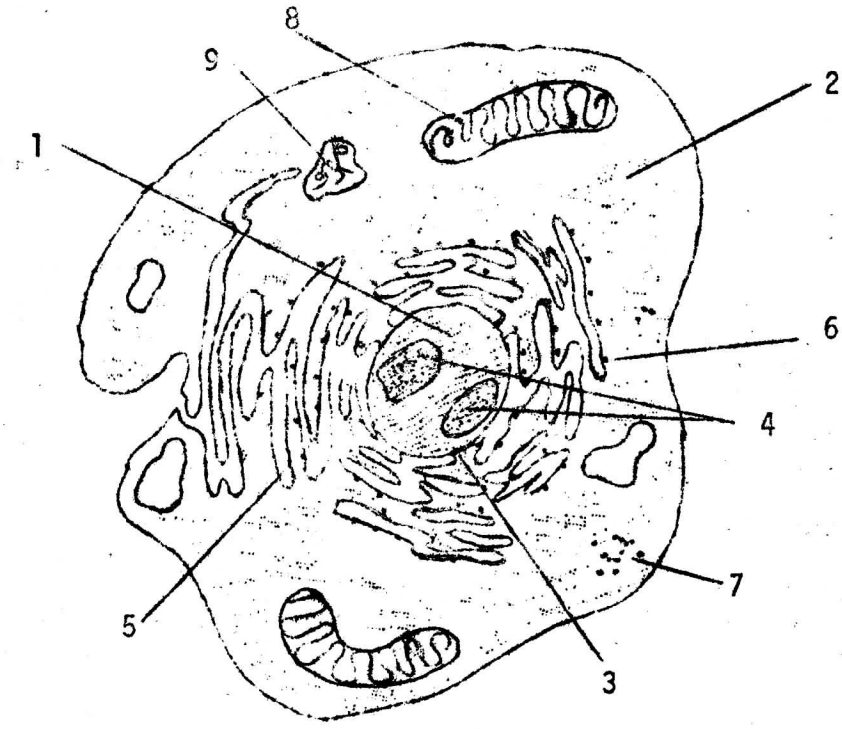

Figure 1: The Generalized Cell (from 14): *The cellular substance is generally referred to as protoplasm. It is subdivided into the* (1) nucleus *and the* (2) cytoplasm. *The nucleus is the largest distinct singular component of the cell. Denser than the cytoplasm, the nucleus is surrounded and contained by the thin* (3) nuclear membrane. *The nucleus contains the chromosomes and chemically regulates the cell processes. Also within the nucleus are the* (4) nucleolus *which are considered the site of RNA synthesis. The* (5) endoplasmic reticulum *is a system of membrane-limited channels which serve as a bridge between the nucleus and the cytoplasm at large. The* (6) ribosomes *are associated with the endoplasmic reticulum and serve as the site of protein synthesis. The* (7) proteins *(amino acids) are the basic structural building blocks of most of the components of the cell. Further, certain protein molecules are enzymes and as such are catalysts of the vital chemical reactions and other biochemical activity of the cell.* (8) Mitochondria *are centers of cellular respiration and metabolism. They are the power house of the cell. The* (9) lysosomes *store and release enzymes which break down food particles within the cell. These enzymes can also dissolve the entire cellular structure of dead cells.*

considered to be the chief site of cryoinjury (8,15,16,17,18,19: abstract 24); the specific mechanisms will be discussed later.

Cellular protoplasm is a viscous, colloidal system which is ever changing to the varying environment and conditions. The cytoplasm consists of all cellular substance outside the nucleus. The cytoplasm contains a mesh of membranes and membrane limited spaces which are observable only under an electron microscope. This membrane system is vital in the internal osmotic process of the cell. Within the cytoplasm are found many cellular organelles, enzymes, and molecules (14) which contribute to the biochemical functions of the individual cell (see Figure 1).

The cell, with its organelles, or subunits, and biochemical activities, could itself very nearly qualify as an organism. Its structure is the key to its successful functioning. Alter the superstructure of the cell, and one alters its function. Correspondingly, if the function of cells within a system (i.e., a tissue, organ, or organism) is altered, the successful function of that system will ultimately be altered accordingly.

MECHANISMS OF CRYOINJURY

With some understanding of cellular superstructure and the interactivity of cell organelles, we can proceed to discuss the means by which the structure and function of the cell are damaged during freezing.

Intracellular water generally does not crystallize until about -10°C to -15°C (15,20,21). This is true even when ice has formed extracellularly. As a result, the intracellular water is at a temperature below its freezing point and is said to be "supercooled". Supercooled water has a vapor pressure higher than that of ice (21). This vapor pressure gradient forces the cell to attempt equilibration. The phenomena which occurs as cells equilibrate depends primarily on the cooling velocity employed and membrane permeability to water. Cooling velocities have been the subject of study for some time.

In 1949, Smith and her associates determined that 1°C per minute was the optimal rate for cooling bull spermatozoa (3). Until recently, this cooling rate has been the accepted practice of cryobiology. More recent work (8,15,22) has shown, however, that this cooling velocity can lead to failure when applied to other cells or tissues.

A cooling velocity which is faster than optimal results in the formation of intracellular ice, or rupture of the cell membrane due to equilibration (osmotic) pressure gradients. Cooling rates less than optimal result in solution damage and other types of damage linked primarily with dehydration of the cell (these are discussed more fully below).

The optimal rate for a given cell would lie between these two extremes (1,10). For most nucleated mammalian cells, no true optimal rate seems to exist (7,15,23). In many cases, however, slower cooling of the tissues, using a cryoprotective agent, to at least -70°C with subsequent rapid rewarming seems to be more conducive to tissue survival than other cooling/thawing velocity combinations tried (5,23,24).

If the cell is cooled slowly or if its permeability to water is high, it will equilibrate by giving up intracellular water to extracellular ice formation. In other words, the cell dehydrates. On the other hand, should the cooling velocity be rapid or membrane permeability be low, the equilibration will be effected by intracellular ice formation.

Mazur (15) and Lovelock (25) suggest that slow freezing leads to solute damage. As dehydration within the cell progresses, solutes precipitate out (i.e., the ice crystals contain no solutes) and the high concentration of electrolytes left behind damages the cell. Mazur (15,19: abstract 25) proposes that another possible cause of slow freezing damage is the severe reduction of cell volume. Progressive membrane stress is caused as this volume reduction surpasses tolerable limits.

In a more recent work, Litvan (21) describes the consequences of dehydration as being the denaturation of proteins, the building blocks of the cell. His hypothesis is that dehydration continues gradually even down to -60°C, which is well below the eutectic temperature for most salt solutions. The decreasing amounts of water within the cell causes the protein molecules to lose their protective layer of water. This results in denaturation of proteins and further decreases in cell volume.

Rapid cooling rates, according to Mazur (15), lead to the crystallization of intracellular ice before the water can be drawn out of the cell through the plasma membrane by osmotic pressure. Intracellular ice, in most cases, alters the structure of the plasma membrane and cell organelles as it expands during the phase change. There have, however, been reports (8,17,26,27) which have demonstrated that intracellular ice is not necessarily lethal in all cases.

Litvan (21) observed cases where intracellular ice did not precede or (he extrapolates) cause cellular destruction, but followed it. Based on these observations, he proposes that cryoinjruy is not due to intracellular ice as much as to membrane rupture by other causes. During rapid cooling, osmotic stresses tend to remove water from the cell at a rate which surpasses the permeabiltiy of the cell. This tends, according to Litvan, to either leave large holes or breaks in the membrane, or to total membrane destruction.

MECHANISMS OF CRYOPROTECTIVE AGENTS

Relevant to our discussion of cryoinjury are the mechanisms by which the cryoprotective agent (CPA) prevents damage. These agents are classified as either penetrating or non-penetrating (10,15,21) depending on their ability to pass through the cell membrane. Penetrating agents are usually associated with the prevention of slow freezing damage while non-penetrating agents are most effective in cases of rapid freezing (1,10).

By foreseeable techniques, the induction of solid state hypothermia in whole organisms, especially large mammals, cannot be accomplished at high cooling velocities. We will, therefore, be more concerned with the prevention of cryoinjury which stems from slow cooling rates. Let us then consider the cryophylactic properties of penetrating agents first.

The most often used cryoprotectants in this group are dimethyl sulfoxide (DMSO), and glycerol. Also included in this group are methyl formamide, dimethyl acetamide, ethanol, and methanol. The rate at which different compounds of this classification penetrate cells varies widely and is due not only to the characteristics of the compound, but also to the type and origin (animal) of the cell (10), as well as temperature and other parameters.

As stated, intracellular ice formation (resulting from rapid freezing) may not be totally incompatible with the viability of all cells under all circumstances (8,17,26,27). The results of slow freezing appear to be far more problematic, and it is on these mechanisms that most penetrating cryoprotective agents are most effective.

One objective in the use of a CPA is the minimization of the vapor pressure differential (between the aqueous solution and the solids) within the cells. This can be accomplished by either lowering the vapor pressure of the intracellular fluid or increasing the vapor pressure of the extracellular solids (21). Cryoprotective agents can assist in either case.

The aqueous solutions of these penetrating agents are low in vapor pressure, and their introduction into the intracellular solution will result in a lowered vapor pressure of that solution. The vapor pressures of vitrified (glassy) extracellular solids are higher than the crystalline forms (ice). Vitrification takes place below -120°C in water and in most substances with a high viscosity (5,21). It therefore seems significant that most cryoprotective agents are found to be highly viscous, especially at low temperatures. There are further implications of this viscosity which will be discussed later.

Additionally, the CPA affects the eutectic point of the solution, lowering the temperature of crystallization in relationship to the amount of the CPA found in the solution. This supercooling allows the aqueous solution to reach the temperatures required for vitrification before solidification takes place.

As stated, the cooling rate and membrane permeability are two important factors relating to the manner in which cells equilibrate as a result of the vapor pressure differential. Both of these parameters have problems of their own associated with them. We discussed the fact of optimum cooling rates and the result of cooling velocities which are less than optimum; the greatest damage usually resulting from slower than optimum cooling rates. The CPA helps minimize this damage in several important ways.

Penetrating and non-penetrating CPA's alike seem to affect the permeability of the cell membrane such that larger molecules may pass through. This will be discussed in more detail under the discussion of non-penetrating agents. One mechanism by which penetrating compounds such as DMSO protect cells is by limiting solute damage (4,9,15,19: abstract 25). This protective mechanism has been referred to as similar to that of antifreeze (19:abstract 25), reducing the amount of ice formed extracellularly. This then limits damage due to high electrolyte concentrations in two ways.

First, if extracellular cystallization is minimized, then the extracellular concentration of solutes is likewise held within more tolerable limits. Secondly, extracellular ice causes the vapor pressure gradient across the cell membrane which promotes cell dehydration through osmotic pressure. As less water "freezes out" of the cell, the electrolyte concentrations left behind are less concentrated.

In addition to minimizing solute damage through an antifreeze mechanism, the penetrating agents are helpful in still another way. Meryman (9), Lovelock (4) and Farrant (12) all propose that penetrating agents protect on a molar basis, by reducing the electrolyte concentration in the unfrozen solution both in and around the cell at any given temperature.

If a nonelectrolyte such as DMSO or glycerol is present, the electrolyte concentration will be reduced by dilution and the extent of the reduction at a given temperature will be approximately proportional to the osmolar ratio of the CPA to the electrolytes in the initial suspension. Only low molecular weight solutes which readily absorb moisture reduce the high molecular weight of a solution. The additive must also have the ability to permeate the cell membrane.

Fishbein (28) states that in the case of DMSO, he found that the intracellular accumulation of this agent alone did not seem to be sufficient for cryophylaxis. This brings us to the question of cell volume. The

exchange of the protective compound for cellular water appears to be required before protection is effected. Shlafer and Karow (29) observed no cell volume changes after treatment with DMSO on cardiac tissue. They assumed that the compound displaced and substituted for intracellular water after osmotic equilibration was achieved. This, then, acts to limit the dehydration process and keeps membrane stress due to shrinkage to a minimum. DMSO (and the more penetrating agents) cause loss of cellular water without apparent loss of liquid volume. The author has found no reference which attributes this same "volume maintenance" characteristic to glycerol. Perhaps this is due to the fact that glycerol permeates cells so slowly, and in some cases (10) even seems totally unable to permeate certain tissues at all.

The rate of penetration by the CPA does not, however, equal that of water. As a result of this, the addition of protective compounds to, and their removal from specimens poses the very real danger of osmotic damage or dehydration to the cells (5). This danger is, of course, far greater in intact organs and animals where perfusion is necessary, than in cell suspensions and tissues.

Some compensation for this osmotic dehydration prior to the full equilibration of the CPA into the cells is achieved (21) as a result of increased intracellular viscosity. The more viscous the solution, the more the flow of water out of the cell is retarded. This increased viscosity is produced by the continuing buildup of CPA within the cell. As stated previously, DMSO and other penetrating protectants are known to have high viscosities, particularly at low temperatures.

Cells may also avoid exceeding their minimum tolerated volume, even without penetrating agents, by permitting an influx of extracellular solution as osmotic stress develops (5,19: abstract 25). This influx includes some intracellular constituents from surrounding (dying) cells. Intracellular substances, including proteins, are released as membranes rupture and may have some limited beneficial effect (1) on the cells which eventually do survive.

Molecular polarity and the ability to easily form hydrogen bonds seem to be characteristic of the more effective cryoprotective agents (30). Once the water is bound, it is less readily available for ice crystal formation. This, again, aids in the retardation of vapor pressure gradients and dehydration. Further, observations (5,21) have confirmed that at cryogenic temperatures (these references are not specific as to the exact conditions under which this occurs), the ice which finally forms is "glassy" (vitreous) and noncrystalline. This alleviates the concentration as well as the dehydration problems since, when intracellular water vitrifies, the solutes are not left behind (5). It is also interesting to note that no crystalline ice forms in DMSO solutions above 40 percent concentrations (21).

Although the protective qualities of penetrating cryoprotective agents can be attributed primarily to colligative effects, the protection of non-penetrating macromolecules can not (4). The protection of these latter compounds is not well understood. In 1954, Lovelock (31) stated that the results of his observations indicated that protection could occur only when the agent penetrated the cell. Meryman (9) substantiated this view. It is now known, however, that penetration is not in all cases required to introduce some (if limited) degree of protection (1,8,10,21) by macromolecules and other non-penetrating CPA's such as sucrose, dextran and other sugars, polyvinylpyrrolidone (PVP), hydroxyethyl starch (HES), etc.

One mechanism by which the non-penetrating CPA protects cells is by increasing cell permeability (10) and thus allowing the cell to "leak" solutes reversibly under osmotic stress. Increased membrane permeability seems to be beneficial in that it permits higher cooling rates to be utilized without causing membrane injury, i.e., reducing the extent of damage at any given temperature (21). Williams and Meryman (10) have demonstrated increased permeability of cell membranes in spinach during the increase of "winter hardiness".

Perhaps the cellular susceptibility to freezing damage is dependent as much on surface, membrane protection as on interior protection? Perhaps the cell interior receives some protection by the high concentration of macromolecules normally within it? These are questions not yet satisfactorily answered.

SUMMARY

As we have seen, the proposed mechanisms of cryoinjury which are prominent in present theory can all be minimized, perhaps even totally avoided in cell cultures or tissue samples, by the use of cryoprotective agents. The properties and actions of the CPA which have been found to be critical are:

1. Low vapor pressures -- minimizing vapor pressure gradients in intracellular solutions they enter.

2. Lowered eutectic point -- allowing vitrification (non-crystallized solidification).

3. Retardation of extracellular ice formation and minimized osmotic withdrawal of intracellular water which leaves behind high electrolyte concentrations.

4. Nonionized and neutral in aqueous solutions -- limiting electrolyte concentrations on a molar basis.

5. Penetrates cell membrane easily -- displaces intracellular water to maintain cell volume.

6. High viscosity -- adding to ability to form glassy ice and to retard rate of cellular dehydration.

7. Molecular polarity -- hydrogen bonding ties up H_2O and makes it unavailable for crystal formation.

Basically, the cryoprotectants presently being used are more effective at preventing slow freezing damage (dehydration, solution effects, vapor pressure gradients) than rapid freezing damage (intracellular, crystalline ice). On the whole, the penetrating CPA is more effective than the non-penetrating type. The non-penetrating agents may be valuable additives to penetrating agents, though. If the organism is to be taken to a solid state, however, intracellular solidification will result eventually regardless of the freezing velocity. Vitrification (glassy, non-crystalline ice) made possible by the proper use of cryoprotective agents, may even render this solid state compatible with cell viability. A more complete knowledge of the manner in which cryoprotective agents protect the cell against cryoinjury and better methods of utilizing their properties would lead to greater success in cryopreservation of whole mammals.

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THE PERMANENT CRYOGENIC STORAGE FACILITY

by Joseph G. Cannon

Consulting Engineer

The author is a 57 year old consulting engineer who has been interested in cryonics since late 1964. In 1966, due to lack of interest by cemeteries in providing permanent cryogenic custodial care, he began the pioneering of an enduring organization and facility competent to assume and assure permanent custodial care of the cryogenically preserved. He obtained the first legal opinion confirming the legality of cryogenic interment, started construction of the first cryogenic storage facility, arranged with the Cryonics Society of Michigan for the first standby service during surgery in case of operative failure (his wife the patient), introduced the first legislation to encompass cryogenic interment, organized the first non-profit cryogenic cemetery association and formed a corporation to furnish cryogenic hardware.

If the cryonics concept is to hold rational promise, many factors must be considered, provided for and fulfilled. As with a series of stepping-stones, each step must be achieved before progressing to the next, and all steps must be accomplished to produce the desired result. Thus, it is meaningless to hypothesize as to the relative importance of the various factors.

Nevertheless, the permanent storage facility is unique, for all other areas of concern are of a relatively temporal nature when viewed against the spectrum of time. To fulfill its purpose and discharge its obligations, the permanent storage facility must function without interruption far into the future -- through extensions in time that can be most meaningfully measured, not by the years of service of any one generation, but rather by the number of successive generations that will dedicate their entire working lifetimes to the servicing and management of the facility.

The fact that only one out of ten corporations survives for even ten years should amply demonstrate the staggering responsibilities incurred when offering and promising perpetual custodial care for the cryogenically preserved. Those who place themselves, or dear ones, in the custody of the facility have every right to expect the highest degree of proficiency in all phases of the facility's operation for their hopes are totally dependent on its uninterrupted functioning. Truly, the storage facility assumes an awesome responsibility that demands it be developed and operated with the most meticulous zeal so as to best enhance the odds favoring its perpetual operation.

As with a lifeboat, the best assurance of the facility's successful extended voyage through time will be achieved by giving careful consideration to all the known perils and providing flexible but adequate means for meeting and surmounting unforseeable crises that will arise. What type of organization is most likely to endure through centuries so as to provide uninterrupted service for the cryogenically preserved?

Although it may be possible for one person to develop the physical plant of a facility, procure the necessary cryogenic hardware and supplies and operate it, it would be presumptuous of any single person to attempt to do so for every man is mortal -- a fact sometimes overlooked even by those interested in cryonics.

In this dawning era it may be necessary for a single person to pioneer the development of a storage facility, but its ownership should be turned over to an organization prior to the start of its operation, i.e., prior to the time any cryogenically preserved person is taken into custody for perpetual care. The hopes and aspirations of those who have chosen cryogenic preservation should not be dependent on the future fates of any one, or even several persons, but rather, on a plurality of members of a management group composed of an adequate number of persons to best assure continuity of performance.

A corporate structure might seemingly fulfill this requirement. However, in many instances, the laws and regulations governing the operation of a corporation are not specifically tailored to necessarily enhance the longevity of the corporation's existence.

The one type of organization that is legally constituted to incorporate longevity of operation is a cemetery organization. Since it is created to provide a perpetual custodial service, i.e., a public or social service, its organization and operation are carefully prescribed, regulated and supervised to best assure responsible performance in fulfillment of its obligations. Because of the service it renders, it is favored by statute in numerous ways.

While it is possible to form a profit-making cemetery organization as a corporation, the introduction of a profit motive cancels some of the

tax-exemption advantages, could cause an increased probability of internal frictions and, with a new endeavor such as cryogenic interment, could make the operation suspect.

Therefore, it would seem a non-profit cemetery organization should be the most irreproachable and enduring. It is the type of organization that enjoys fullest benefit from the privileges accorded it by society and law. Its plants and lands are tax exempt. Its trust funds and its perpetual care funds are both tax exempt and exempt from the laws against perpetuities. Operating without profit incentive should minimize managerial frictions and enable it to offer cryogenic perpetual care at the lowest feasible cost.

Due to the wide variance in state laws and both state and local cemetery regulations, it is beyond the scope of this article to submit specific recommendations or detailed specifications that would be helpful in the development of a cryogenic storage facility. However, some of the general areas of concern that should be given meticulous consideration are as follows:

1. Topographical Location -- The facility should be located on high ground having unlimited drainage capacity so as to preclude all possibility of flooding regardless of the most inclement weather.

2. Geographical Location -- The facility should be located so that it is accessible at all times regardless of weather conditions, in case of emergency. The location should be such that liquid nitrogen can be obtained at an attractive price from a primary source of supply and within a reasonable distance from a secondary or emergency source, so that transportation costs will not be higher than necessary.

3. Contracts for supplying liquid nitrogen should be made with a primary and a secondary source of supply so that availability of nitrogen on demand is assured.

4. A liquid nitrogen emergency supply stored in a tank or in an extra capsule should be on hand at all times.

5. Maximum security should be maintained at all times. All security devices should operate on closed circuits to assure activation of the circuits. Backup security systems are advisable. Vital alarm systems, such as low nitrogen level alarms and the gauges that activate them should be redundant for high reliability. A custodian should be in attendance at all times and should check all vital alarm

gauges and security systems at frequent intervals so as to assure their proper functioning. Provision should be made to assure the custodian is performing his duties. A periodic call-in system to a remote location is advised.

6. An emergency supply of water for fire protection and an emergency pumping system to keep pressure on the water system in case of failure of the normal water supply is recommended. Means for providing emergency electricity for ventilation blowers, lights and the emergency water pump, etc., is recommended.

7. The board of directors, or trustees, should be comprised of the largest practical number of members to assure continuity of management. Each office should be held by a succession of members of the board of trustees or directors during their tenure on the board to assure a duplication of expertise at all times.

8. The fee for custodial care must be carefully calculated as it will be difficult, and in time impossible, to obtain additional funds for those already in custody. Too high a fee will deny the possible benefits to many. Too low a fee will jeopardize the security of all. Still, the calculations must provide for unforeseen contingencies. A fractional part of the perpetual care trust fund's yield must be retained within the fund and added to it to provide protection against increasing operational costs due to inflation.

9. All trust moneys must be bonded against loss -- with careful consideration being given to the reliability of the bonder.

The preceding enumeration of considerations is not, in any way, intended to present all of the factors to be considered, evaluated and incorporated in the development of a permanent cryogenic storage facility to operational status. However, it is hoped that the points mentioned will stimulate further thought regarding the drafting of adequate provisions to best assure perpetual operation of the storage facility.

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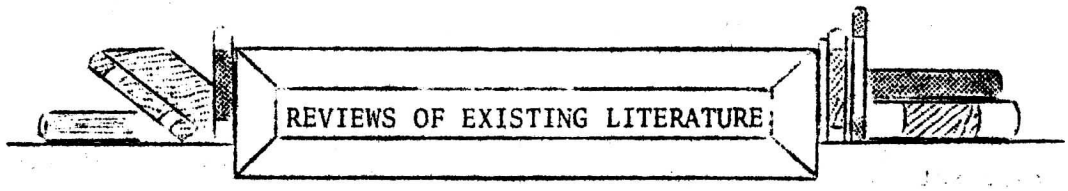

human aging mystery

Many theories exist as to the cause of the aging process. There is increasing recognition that human aging is the result of a number of separate mechanisms operating simultaneously. This paper presents a lay level discussion of many of the more widely accepted theories as to the causes of aging as well as the many problems involved with aging research.

["Human aging: the enigma persists", Howard J. Sanders, Chemical and Engineering News, July 24, 1972.]

The desirability of greater research on the retardation of human aging, according to the author, has found increased awareness among today's scientists. Federal support of aging research has increased and the quality of research has improved in the last decade.

Nine of the better accepted theories of aging are briefly discussed. The "genetic clock" theory holds that heredity is the key to the dilemma of why some species have longer life spans than others. Perhaps there exists "aging genes". The fact of limited cell division was only discovered in 1961. Some cells of the body do not divide at all, such as neural cells, while other cell types have varying, but usually limited, cell divisions. Eventually, many types of cells stop subdividing and their numbers begin to dwindle, resulting in a reduction of the organism's capability for survival.

DNA deterioration is thought to be a possible cause of aging. With time, the mechanisms for repair of DNA molecules cease to function properly. Similarly, another possible cause of aging is the error theory. Due to defective DNA, abnormal proteins become formed which with time inhibit the normal process of the cell.

The break down of the immunological system is receiving more attention as a possible cause of aging. The body, losing its ability to form antibodies fast enough, or unable to form antibodies at all, becomes increasingly susceptible to dangerous bacteria, viruses and foreign bodies. At the same time, the body will some times produce antibodies incapable of descriminating between normal, desirable substances and dangerous substances. This results in the break down of proteins, enzymes, and other useful substances within the body.

Free radicals (molecular fragments) can be involved in various oxidative reactions which can lead to deterioration of lipids, collagen, elastin, and other substances. This deterioration results in impaired cellular functions. The cross-linkage theory holds that the progressive cross-linking of proteins or nucleic acids clogs the cell and, again, inhibits cell function. The effects of cross-linking are striking in connective tissue -- one of the most noticeable symptoms of old age is the change which takes place in aging skin.

Not all scientists agree on these theories. Some see these hypothesized causes as merely the results of aging rather than the causes. No theory of human aging and no experimental evidence has yet resulted in major practical or empirical methods of retarding the aging process in humans. Gerontologists are optimistic, however, that real results will begin to be seen within our lifetime. Some even predict increased lifespans and workable aging therapies well before the year 2000.

LLC

Φ Φ Φ

aging therapies prove helpful

In the past decade, Russian gerontologists have made an intensive study of the effects of several controversial "biologically active substances" which are claimed to retard human aging. This paper discusses some of the published results.

["Study Indicates Vitamins and Other 'Biologically Active' Substances May Prevent 'Premature' Aging", Geriatric Focus, p.2, September, 1972.]

Hundreds of normal, healthy, elderly and aged individuals free of obvious disease (except atherosclerosis) participated in a study at the Institute of Gerontology of the USSR Academy of Medical Sciences. The study included the effects of several biologically active substances on the aging organism. Some of the findings of this study have been reported by Professor D. F. Chebotarev, Director of the Institute.

Gerovital (2% procaine), para-aminobenzoic acid, vitamin therapy, anabolic hormones, placenta extracts (tissue therapy), antireticular cytotoxic serum (ACS), and Apilacum (a preparation of royal jelly) were all studied at the institute.

These studies produced some degree of positive results from each proposed therapy. The article states, "various geriatric drugs affect not only different body systems, but different elements in each system". The conclusion is that selective influence on different functions within the aging organism is feasible.

The study indicates that a preference should be given to multivitamin preparations. Further studies of the geriatric pharmacology are seen as essential and promising in the search for drugs which will affect aging processes in the human organism.

LLC

Φ Φ Φ

gerolytic enzymes --
cross-linkage digestors

The cross-linkage theory of aging, supported by the authors, has long been under consideration. If enzymes produced by soil bacteria could be found which had the ability to destroy the cross-linkages formed with aging, perhaps a valuable aging therapy could be developed. This paper documents the isolation of such enzymes.

["Study of Low Molecular Weight Proteolytic Enzymes", J. Bjorksten, E. R. Weyer, and S. M. Ashman, Finska Kemists. Medd., 80 N:0 4, 1971, Soumen Kemistis. Tied.]

The report documents the isolation of micro-organisms capable of surviving by digestion of both synthetic materials with the highest order of intentionally produced cross-linkages and insoluble fractions from an 80 year old human brain. Significantly, the fractions from the human brain were more resistant to such digestion than the toughest artificial materials.

The search for organisms capable of breaking down insoluble material from aged human brain tissue was necessarily the first step. The organisms were exposed to a medium containing the substance in question as an exclusive source of nutrient needed by the organism. The authors then cultured three of the most promising organisms and extracted their enzymes.

In the next phase of experimentation, the authors seeded baby rats, before birth, with radioactive materials (by feeding these to the mother), let the rats grow to old age, and then dissolved away everything in the rat brains except the most stubborn material.

This remaining material contained very measurable amounts of radioactive materials, which were bound into the very matrix of the substance. The radioactive counts of the material withstood all of the dissolving techniques of a conventional kind that could be brought to bear. Then the isolated enzymes were shown to be able to digest the substance.

Bjorksten and his coinvestigators continue, now, to pursue the problem of using these enzymes in living physiological systems. The authors state, "we will direct our search toward enzymes characterized mainly by

molecular weights low enough to penetrate cage structures, and compatibility with living systems. The enzymes in hand are being studied further as they represent the best means now apparent to act upon gerogenic insolubles under physiological conditions".

There are probably many serious difficulties to be overcome, but this article is proof that the initial hypothesis of finding very active enzymes with the capability of dissolving the worst cross-linkages was valid.

FRC

Φ Φ Φ

new era in cancer research

Cancer is second only to heart disease in the number of victims it strikes down annually. More is known about preventive measures for heart disease than for cancer. The result is to give cancer a very much more fearful spector. Any new advances, such as those reported below, are welcome.

["Experts Hunt a Mysterious Cancer Agent", Los Angeles Times, Part 1-A, October 18, 1972.]

"New York -- exclusive to the Times from Reuters -- A new era in cancer research is opening in laboratories around the world -- the hunt for the elusive chalone. Scientists are not at all certain there are such things as chalones (pronounced kay-lone), although most admit there should be something like them in living cells. The important thing is that if chalones can be found it might be possible to cure all forms of cancers with serums. It would not be necessary to find the cause of the disease.

"Normal cells divide very slowly if at all. The process of division, or reproduction, is called mitosis. Sometimes, however, cells go wild, reproducing madly. That is cancer. The obvious question is why most cells never go out of control.

Two Substances?

"Some scientists now believe there is something in the cell that inhibits mitosis. They call it chalone, a Greek nautical word meaning to 'slack

"off'. Others think there are two substances, the chalone and an anti-chalone. In a normal cell, the theory goes, both substances are balanced, with the chalone dominant. Cancer occurs when an anti-chalone element somehow gains dominance over the cell.

"The concept grew mainly out of three men: Paul Weiss of Rockefeller University; William S. Bullough of Birbeck College, London, and O. H. Iverson of Oslo, in 1957. They convinced enough scientists so that a convention was held last spring. That in turn produced widespread interest in the idea. It is an attractive one.

Research has indicated that chalones are tissue-specific but not species-specific. That means you could take a chalone from the liver of a pig and use it to stop mitosis in a human liver cell.

Early Progress

"It seems reasonable to believe a serum could be obtained from animals that could stop specific cancer growth in humans just as horse serum has been used to fight tetanus. The easiest cells to work with are skin cells, which never divide when healthy. A researcher in Philadelphia, Dr. Dharam Chopra of Temple University, is using psoriasis cells. Psoriasis is an uncomfortable skin disease, technically a cancer although it is not malignant, and is more unsightly than fatal.

"Serum taken from psoriatic cells-- which would logically be heavy with anti-chalones -- have been introduced into normal skin cells. Chopra says they began to divide. Serum from normal cells -- weighted with chalones -- have no effect. He is convinced a white powder he is extracting from cells contain chalones, although he says he has no idea how they work.

Research Money

"Finnish scientist Tapio Rytomaa took chalones from normal rat cells and rats suffering from chloroleukemia, a fatal blood cancer. The chalones were injected into the sick rats. The life expectancy is 12 days. Three rats are still alive three years later and the lives of all the treated rats exceeded the normal.

"Its now believed possible that cancer occurs when the chalone substance -- believed to be a protein -- breaks out of the cells and into the blood stream faster than usual. That may account for the fact that cancerous animals appear to have a high chalone content in their blood.

Convinced the chalone advocates may be on to something, the National Cancer Institute has awarded its first grants in chalone research. 'The field of cancer research', Bullough said, 'is not so rich in ieeas that it can afford to ignore this one'."

Φ Φ Φ

films available

The UC Berkeley film catalog offers the following films. Many other films of diverse subjects are also available. These films, as well as others of interest, may be available at most other college and university libraries around the country.

AGING: THE SEARCH FOR ETERNAL YOUTH # 8425 Towards the Year 2000 Series col 22m r$25

Discusses problems of aging in our society and considers possibilities for control and even reversal of the aging process.
rd1972 DA.

BIOCHEMEDICAL REVOLUTION: MOODS OF THE FUTURE # 8405 Towards the Year 2000 Series col 22m r$25

Dr. Donald Luria, drug expert, examines prospects for drugs that affect such things as intelligence, memory, and aging.
rd1971 DA

BRAIN: CREATING A MENTAL ELITE # 8402 Towards the Year 2000 Series col 22m r$25

Arthur C. Clarke and several scientists consider three major areas of future brain research: chemical and electrical stimulation, and environmental conditioning.
rd1972 DA

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Question: In section 56.0 of the instruction manual, the function of most of the cannulation instruments is described, however, a section on the use of forceps is not included. Could you supply this description as well as more information for purchasing forceps?

Answer: A future revision of section 56.0 will contain the following information: Forceps are used primarily for two purposes (a) manipulating tissues and ligatures, such as when raising the vessels, and (b) "spreading" veins to facilitate the removal of clots during the Phase I procedure of replacing the blood with perfusate. Although section 56.2.2.6 describes the use of a drainage tube for removing clots, it will sometimes be necessary to remove that instrument and allow large pieces of coagulated material to pass through the opening in the veins by enlarging that opening using spring-action forceps. One mortician has suggested that two forceps (one each of two different styles) may be adequate: one straight (5 to 8 inch length) and one curved (7 to 12 inch length). These forceps list for $1.60 to $6.10 from Kelco Supply Company (see Appendix A of the manual).

Question: In figure 56-3 (section 56.0 of the manual), only two sets of arterial cannulas are shown as being required. But if they are used to make the "T" in both the artery and the vein, wouldn't four be needed?

Answer: Yes, should conventional cannulas be used to set up a "T-shaped" flow configuration, four cannulas will be required.

The number of hemostats recommended in Figure 56-3 should also be increased. Since the hemostat is so universally useful, it may, in some cases, prove valuable to have six hemostats.

Question: Section 56.0 of the manual recommends a "thread passer", but does not discuss its purpose. Please describe.

Answer: The thread passer is an instrument which contains the ball of thread (used for ligating) and is equipped with a pointed extention which can be passed under the raised vessel (see 55.2.2.3 of the manual). The thread passer currently lists for approximately $14.00 (Kelco Supply). The procedure can also be easily accomplished with forceps or aneurism hooks.

Question: Figure 58-1 in the instruction manual calls for 0.25 grams or 3.86 grains of phenoxybenzamine per liter in the Phase I perfusate. Is this amount correct?

Answer: The amount of phenoxybenzamine shown in Figure 58-1 is incorrect. The correct amount should be .025 grams or .386 grains per liter. Other corrections to Figure 58-4 are as follows:

COMPONENTS	CHANGES
$NaHCO_3$	eliminate altogether
$MgSO_4 \cdot 7H_2O$	5.72 grains/liter and 86.6 grains/4 gallons

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--EDITORIAL-- (continued)

More about Alcor will appear in future MTR issues, as previously stated. Again, other cryonics societies are encouraged to prepare and submit articles detailing their facilities, training programs, equipment, etc. Only by an open exchange of this type information can our "state of the art" be pinpointed and then brought to its highest potential of development.

F. R. Chamberlain

1. "Cryonic Suspension Mobile Unit", Walter E. Runkel, *Manrise Technical Review*, 2:90-4, 1972.
2. "New California Organization", *The Outlook*, 3:8, 1972.

The Manrise

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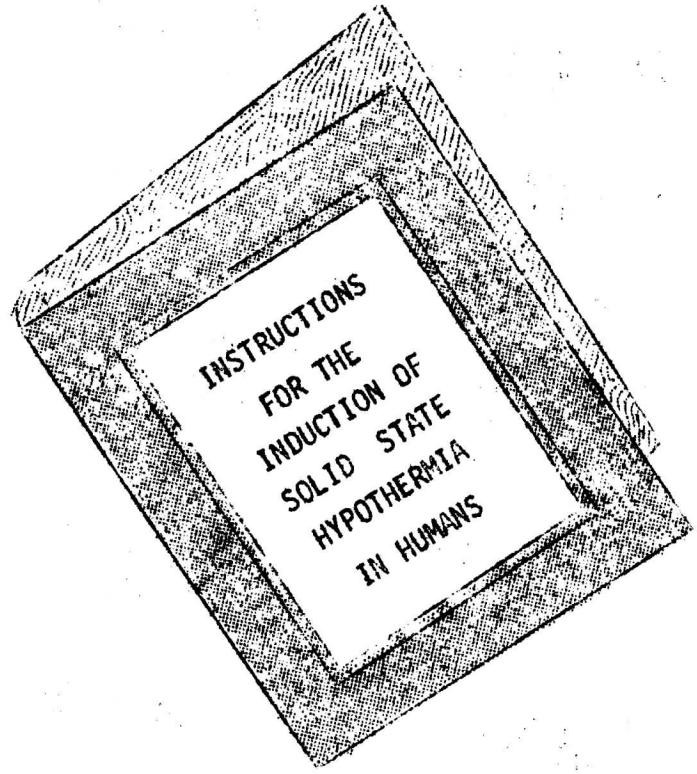

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