Liquid Nitrogen: Its Cryogenic Properties and Unique Healing Process

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Executive Summary

- Liquid nitrogen has a long history of medicinal use dating back to the 1950's
- Liquid nitrogen's unique cryogenic properties, which have been exploited for external human use, predominantly in dermatology, arise from the rapid and deep freeze that occurs when liquid nitrogen touches living tissue
- Such cryothermic treatment results in a non-scarring regenerative/rejuvenative healing response rather than a more fibrotic, scarring, reparative healing process that is a common result of hyperthermic therapies (cautery, radiofrequency ablation, laser)
- Liquid nitrogen spray allows delivery of this cryogen in a non-contact manner
- The use of liquid nitrogen on the lining of internal organs has been made possible by controlling liquid nitrogen delivery through a catheter using the working channel of endoscopes and bronchoscopes
- Liquid nitrogen's extreme cold destroys tissue while preserving the extracellular matrix

History of Liquid Nitrogen

The general applicability of cryogen therapy is underscored by over 30,000 cryosurgery and cryotherapy publications listed in PubMed with a recent and significant upward trend. (Figure 1). The use of liquid nitrogen was generally restricted to application via a cotton swab until the advent of modern cryosurgery began with Cooper's development of a closed system liquid nitrogen probe.[1] Since that time many different devices have been developed including spray forms of liquid nitrogen spanning a large range of applications including dermatology, oral surgery, gynecology, urology and general surgery. Liquid nitrogen has, until the recent development in the early 2000's of a low-pressure catheter-based spray system[2], been delivered topically either externally or via open surgical procedures. **"**There are a number of advantages to cryosurgery for the destruction of tissue as compared to other methods of treatment, most of which arise from the fact that no tissue is excised. Rather, cryosurgically treated tissue is left in situ and allowed to become necrotic… the advantages include simplicity of use, the need for little or no anesthesia (because the extreme cold has an analgesic effect), avoidance of hemorrhage and relatively few postoperative complications.**"**[3]

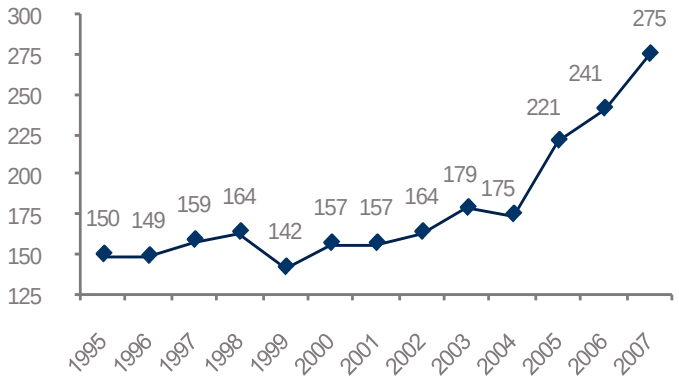


Figure 1 U.S. National Library of Medicine

Cryoprobes and the Joule-Thomson Effect

Most cryoprobes utilize a compressed gas that is suddenly allowed to expand into a closed space (typically the tip of a cryosurgical unit). This sudden gas expansion causes a drop in temperature at the cryoprobe tip, and the freezing of adjacent tissue occurs. This process is commonly called the Joule-Thompson effect. Typical compressed gases utilized are nitrous oxide and carbon dioxide.

When using cryogens via a probe tip, temperatures of -40 to -89 degrees Celsius are routinely achieved, making such cryogens amenable to treating benign and inflammatory diseases and some forms of precancerous lesions. Although

such cryosurgical units are common, a major drawback of use is the direct contact that they require to the tissue undergoing treatment. In order to avoid damage to the treated tissue, the physician must wait for adequate thawing in order to atraumatically remove the tip from the tissue surface.[1]

Characteristics of Liquid Nitrogen Freeze Effect (Hard/Snap/Flash Freezing)

Although liquid cryogens, such as liquid nitrogen or liquid oxygen, have very low boiling points, liquid nitrogen has the lowest at -196 degrees Celsius. This extremely low boiling point allows liquid nitrogen to be used for the treatment of both benign and malignant diseases – it also leads to a very quick transition of the liquid to a gaseous state, thus absorbing energy in the form of heat from the adjacent tissue. Typically these cryogen liquids are delivered via a non-contact method such as spray, a direct improvement over cryoprobes.

In general, the mechanism of action for tissue destruction by cryogens is based on the development of ice crystals. Given that cells are approximately 70% or more water, cold exposure will lead to ice crystal formation. Some of the defined steps of cell death due to cryogen exposure are:

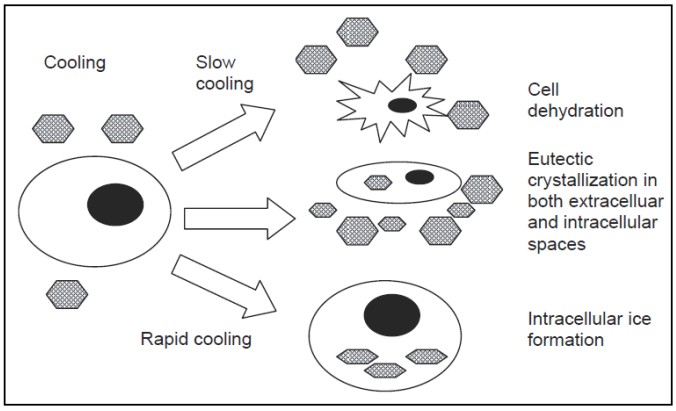


Figure 2: Diagram of the mechanisms of cryotherapy effect on cells.[3]

1. Development of intracellular ice formation.
2. Development of extracellular ice formation.
3. Cell dehydration with cell shrinkage.
4. Abnormal concentration of electrolytes within the cell.
5. Thermal shock.
6. Denaturation of lipid-protein complexes.

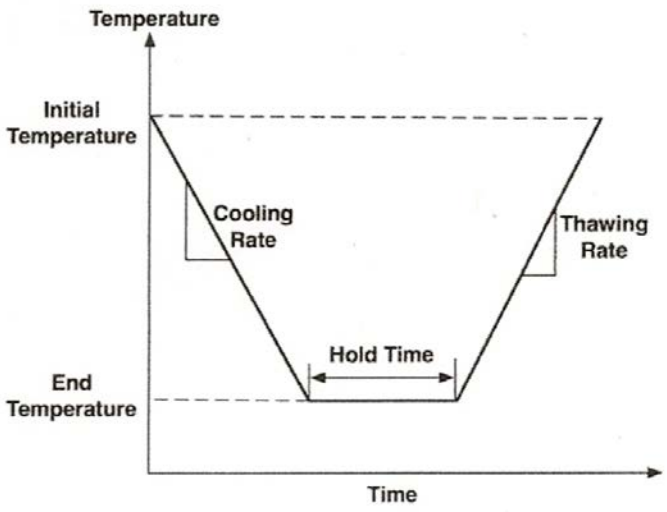


Figure 3: Diagram of cryogenic temperature model.[4]

Most cryogens applied via cryoprobes use the development of extracellular ice to kill cells. This process is typically referred to as a "slow freeze" - cooling defined as a drop of less than 100 degrees Celsius/minute. Water is drawn out of cells by the change in extracellular osmolality and then held in the extracellular space by the creation of ice crystals. Cell death is enhanced by allowing passive warming (no active heat is applied to the tissue) making the intracellular/extracellular movement of water more disruptive to cell walls. This slow cooling process indicative of cryoprobes requires the well-known freeze-thaw-freeze approach in order to be effective. Often the dwell time of the cryoprobe exceeds several minutes of direct contact with the treatment area. Full thaw is required to disconnect the probe from the tissue surface.[3]

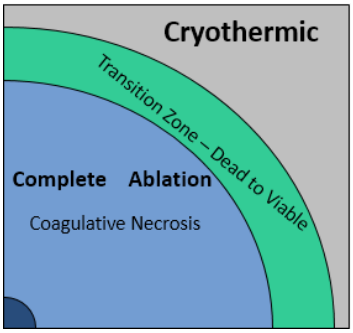
Liquid nitrogen spray creates a non-contact form of cryogen therapy. It is the change of phase from a liquid into a gas at such a low temperature (-196 degrees Celsius) that endows liquid nitrogen with its significant cryogenic effect. Such a vast difference in temperature between liquid nitrogen's boiling point and tissue leads to a rapid freeze, often termed a rapid cooling or a hard, snap or flash freeze - defined as a greater than 100 degree Celsius/minute drop in temperature. Exposure to this significant temperature drop leads to intracellular ice formation, and with passive warming to body temperature, the intracellular ice crystals aggregate causing rupture of intracellular organelles and

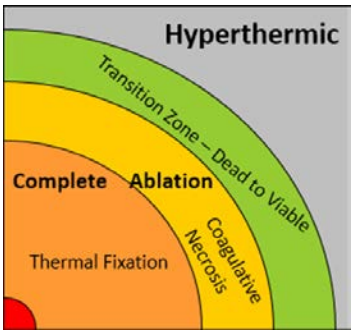
cell death. **Thus a hard freeze leads to more extensive primary cell death over a shorter freeze time, typically in the range of a minute or less.** Often such hard freezing allows the use of a single freeze/thaw cycle to achieve lesion treatment, rather than the repetitive freeze/thaw/freeze process that defines slow freezing.

Keep in mind that a freeze process, whether singular or multiple, will cause the initial cell death. Other processes such as loss of vascular access, metabolic shock, etc., will lead to secondary cell death via apoptosis. These processes are generally referred to as primary and secondary cell death, respectively.

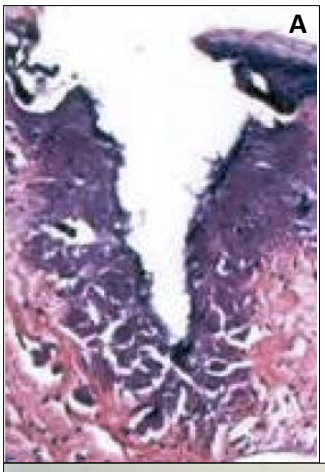
The degree of temperature penetration/loss of blood flow into the targeted tissue will depend on the strength of the freeze (hard being more effective than slow), the length of the freeze (often referred to as the "dwell time" during which the tissue is exposed to the cryogen), and the amount of cryogen delivered over what area. Such parameters are similar to the delivery of laser therapy or radiofrequency-delivered hyperthermia therapy. Eventually equilibrium will be reached between the penetration of the cryogen cold front and the inherent tissue warmth from blood flow.

How Cryotherapy Differs from Hyperthermic Therapy





Depicted in the drawings at left are the basic differences between the hyperthermic versus cryothermic therapies. The source of the therapy is represented by the blue or red semicircle in the lower left corner of each drawing. Emanating from this point is the initial effect of the thermic process. For hyperthermia, the heat creates complete thermal fixation which is the destruction of cellular membranes, intracellular organelles and extracellular matrix. This process creates cellular degradation with loss of typical tissue architecture as noted in Figure 4A below. Beyond this region of cellular degradation is a small band of coagulative necrosis where cell death occurs but the cell membranes are left intact. The transition zone has a gradient of cell kill from mostly killed to mostly viable cells.



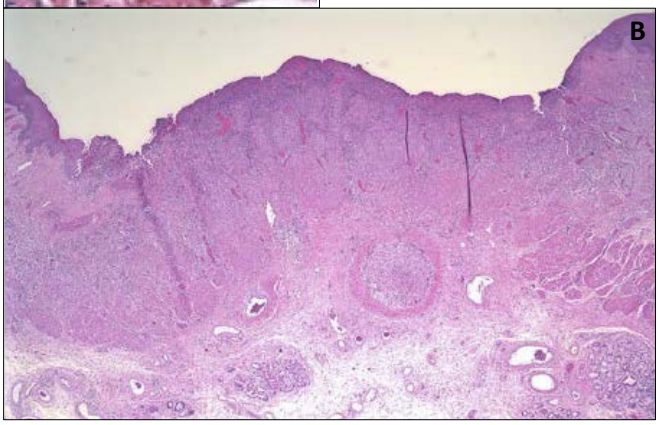


Figure 4: In panel A (left) is a typical hemotoxylin/eosin (H&E) stained tissue section after treatment with hyperthermia. There is marked loss of tissue architecture and general degradation of cellular structure. Panel B (below) represents tissue after treatment with cryotherapy. Note that on H&E staining the overall tissue architecture integrity with normal cellular structure has been preserved.

For cryothermic therapies, the initial field near the cryogen source responds to the thermal effect with complete ablation via coagulative necrosis. As the cold front recedes into the tissue, a transition zone much like that found in hyperthermic therapies occurs with increasing cell viability the farther from the cryogen source one goes. In a cryothermic treatment, particularly in a hard freeze as with liquid nitrogen, the cells remain intact in the field of coagulative necrosis as it is the intracellular organelles (mitochondria and endoplasmic reticulum) that fully disrupt leading to cell death. Thus this process leaves the cells and tissue architecture intact when evaluated by hematoxylin and eosin staining seen in Figure 4B above.

Description of the Healing Response Expected From a Rejuvenative Versus a Reparative Healing Process

Wound healing is a complex and dynamic process of replacing devitalized and missing cellular structures and tissue layers. The human adult reparative wound healing process can be divided into 3 or 4 distinct phases. The most commonly used 4-phase concept includes the hemostasis phase, the inflammatory phase, the proliferative phase, and the remodeling phase.

Names of phases can also vary at the author's preference. For example, the proliferative phase is also referred to as the granulation phase, and the remodeling phase can also be called the maturation phase.

Within these broad phases are a complex and coordinated series of events that includes chemotaxis, phagocytosis, neocollagenesis, collagen degradation, and collagen remodeling. In addition, angiogenesis, epithelization, and the production of new glycosaminoglycans (GAGs) and proteoglycans are vital to the wound healing milieu. The culmination of these biological processes results in the replacement of normal tissue structures with fibroblastic mediated scar tissue and often excessive granulation tissue.

The terms regeneration/rejuvenation/remodeling all depict the same type of wound healing. However the term regeneration can be incorrectly interpreted, as adult mammals will not regenerate large structures such as replacing a missing limb. In cryogenetically induced lesions, the extracellular matrix remains intact which readily supports replenishment of the tissue that was treated. This residual scaffolding facilitates removal of the dead material, keeps the tissue aligned, and allows re-epithelialization over a shorter time period and with fewer deposits of cytokines – which would call in additional fibroblasts leading to reparative healing or scar. Often the treatment of abnormal tissue with cryogenic therapy leads to regrowth of normal-appearing tissues without excessive deposit of fibrotic scar.

With spray cryotherapy (SCT) using liquid nitrogen as the cryogen, the hard freeze leads to cellular death without extracellular matrix loss. Cells repopulate the region in accordance with their respective cell layer and in areas that were treated for abnormal cellular growth, the cells that repopulate are representative of the prior normal cell layer.

Summary

Here we have described thermal properties of cryogens, focusing specifically on liquid nitrogen and its unique cryogenic properties. This review addresses how thermal injuries are delivered and how they heal.

References:

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