Practical Cryosurgery

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PREFACE

It is now some 150 years since James Arnott first enthusiastically described the beneficial effects of applying 'severe' cold causing 'benumbing' or 'congelation' in the treatment of a variety of conditions; including headaches, erysipelas, rheumatism, inflammation and regression of malignant disease. Since the advent of modern versatile equipment, cryosurgery as a technique has become an accepted procedure in an equally diverse range of situations covering many specialties. It is for this reason that surgeons with experience in the use of cryosurgery in their own fields have contributed to this book.

In presenting an essentially practical account, one should be realistic and admit that cryosurgery is a simple technique to do, anyone with appropriate equipment can follow the procedures and treat a patient. But because of the simplicity, the results of cryosurgery should not be minimised or spurned, nor should cases be treated indiscriminately, each must be carefully assessed and the application of the freezing properly timed and controlled. This is particularly important with regard to neoplasia in order to avoid misconceptions about a new method of therapy. Only then will worthwhile results be achieved to the benefit of the patient and the satisfaction of the surgeon.

All the contributors join me in acknowledging the help extended to us by the firms who have done so much in the pioneering and development of modern cryosurgical equipment, such as Frigitonics Inc., Dynatech Corporation, Keymed, and especially to Dr Hilton Thomas of Messrs Spembley Ltd.

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H. P. Holden

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CHAPTER 1

HISTORY AND DEVELOPMENT OF CRYOSURGERY

Cryosurgery is a branch of therapeutics that makes use of local freezing for the controlled destruction or excision of living tissues. Although the effects of cold were probably well known to surgeons in the nineteenth century and before, as both a form of physical trauma and as a palliative in painful conditions, it appears that the first account of a clinical application of the technique was by Dr James Arnott in 1851. While working at the Middlesex Hospital he described the direct application of a salt–ice mixture to various body surfaces at −20°C, and the resultant temporary hardening and whitening of the skin was interpreted as freezing. A wide variety of conditions were treated, most of them being non-specific such as headache, thoracic neuralgia, pruritis pudendi and erysipelas, but they also included locally advanced carcinomas of the cervix and fungating breast cancer. Arnott made no claim for a cure but he drew attention to the anaesthetic and haemostatic effect; and in both malignancies he noted regression of tumour growth, with significant alleviation of pain and reduction in local bleeding and discharge.

The introduction of more efficient and practical methods of applying cold led to renewed interest in this approach, but technical limitations were such that its use was restricted to small and superficial skin lesions. Locally applied solid carbon dioxide, liquid nitrogen and a cold air blast were employed for the destruction of benign neoplasms or pre-malignant dermatoses, and good results were claimed in terms of eradication of the lesion and residual scarring.

In 1961, Dr Irvine Cooper of New York described a cryotherapy unit in which liquid nitrogen was circulated through a hollow metal probe,

vacuum insulated except for its tip. With this equipment it was possible, by interrupting the flow of liquid nitrogen, to control the temperature of the tip within the range of room temperature to −196°C. Similarly, as it was a totally enclosed system, it was possible to apply the cold to any point in the body accessible to the probe. The first clinical application of this technique was in the field of neurosurgery, in the treatment of Parkinsonism. The ability to control both the site and the size of the destructive lesion and the combination of anaesthetic, haemostatic and coagulant properties, however, made it an ideal medium for the treatment of neoplasms. This principle, in which a liquid gas is circulated through a closed system, has been the basis for the majority of cryosurgical units, and various probe designs have been introduced for specific surgical gynaecological applications. Equipment of this type is, because of its complexity, expensive, and alternative instruments have been designed in which the cooling process relies on the 'Joule–Thomson effect', namely, that when a compressed gas is allowed to expand rapidly a fall in temperature results. The depth of cooling varies with the degree of compression of the gas, and with pressures of 2,500 lb/in^2 in conjunction with some form of micro-refrigeration pre-cooling, it is possible to attain a temperature similar to liquid nitrogen. In general, however, although the Joule–Thomson equipment offers several advantages in cost, in mobility, and in more accurate temperature control, with the commonly available pressures it is unable to provide the low temperatures that are looked for in many surgical situations.

It has been clearly demonstrated that, although it is true for clinical purposes that the larger the probe size the greater the freezing power, it is temperature that has the greatest influence on the size of the lesion. Moderate freezing to between −50° and −70°C is suitable for small or superficial lesions, but liquid nitrogen temperatures are essential if larger areas are to be destroyed.

Adequate instrumentation is in many ways the most important constraint to the further development of cryosurgery, there being nothing between the expensive and complex liquid nitrogen powered unit and the relatively weak but simple and inexpensive Joule–Thomson unit. With a greater understanding of the cryobiological principles that influence a cryo-lesion it will be possible to define with accuracy the performance that is required for each clinical situation, and, therefore, the most appropriate equipment. The principles are primarily concerned with the mechanisms of the freezing injury, the cellular changes associated with this form of

injury, and the physical properties of the cryo-lesion, topics that are discussed in some detail below.

THEORETICAL CONSIDERATIONS

MECHANISMS OF FREEZING INJURY

The fundamental principle governing cryo-destructive surgery is that living cells are at first injured and, later, die from the effects of the freezing injury, and that this change is uniform throughout the tissues involved. Unlike so many areas of modern therapeutics this principle is totally accepted by clinicians and can be demonstrated with each cryosurgical procedure. It is all the more remarkable, therefore, that there is no universal agreement as to the reason why there is uniform cell death, and so little information about the exact nature of the lethal insult, that is to say, the critical freezing temperature and the optimal rates of cooling and of thawing.

At least part of the reason for this controversy stems from the very extensive information that is now available about the behaviour of individual living cells when exposed to low temperatures. It is well known that cells that are part of a laboratory model, such as in cell suspensions or tissue cultures, may survive cooling to temperatures of from −70° to −120°C, and tumour cells such as sarcoma 37, and indeed some cell colonies, can retain a significant proportional survival after sudden immersion in liquid nitrogen at −196°C. The physical and biological conditions related to environmental cooling experiments of this nature are, by definition, under full control and there is a great deal of accurate information concerning temperatures and thermal gradients, and from this work it is now apparent that it is the rate of cooling and thawing rather than the absolute temperature that is the destructive agent. It is also apparent that individual cells vary in their ability to withstand these stresses and that a few cells faced with temperature insult are capable of undergoing a protective reaction in the form of an immediate morphological change.

It would be inappropriate to suggest that the cause of cell death in cell suspensions or in tissue cultures is fully understood, but the supposed mechanisms include intracellular ice crystal formation, extracellular ice crystals and subsequent intracellular dehydration, denaturation and alteration of the membrane lipoproteins, and a direct cellular inhibition. Of these, it is the formation of the ice crystals that is most favoured as the

ultimate lethal effect. Their action, however, depends on whether the crystals are extracellular or intracellular, whether, by virtue of the size and shape, they can physically injure the cells, and whether or not the process of dehydration by which water is withdrawn from a cell to form the extracellular crystals, with a subsequent intracellular hypertonicity, is such as to produce cell death.

The rate and site of crystal formation in tissues is related to the solute concentration within the tissues, the freezing temperature, and the freezing rate, and it is the last of the three that is of most significance. Slow cooling (about 1°C per minute) is accompanied by the formation of extracellular ice crystals by a process of heterogeneous nucleation. These crystals self-propagate through the extracellular spaces and gradually increase in size, but in spite of local deformity they may not be injurious to the cell. Thereafter, the further effects vary with the individual characteristics of the cell. In the case of small highly permeable cells, water is withdrawn from the intracellular to the extracellular phase and will contribute to the growth of this extracellular ice, leaving only 'bound' water which is not available for crystallisation but which becomes increasingly hypertonic. If the cell is relatively impermeable to water transfer it may remain unaffected for a considerable time until further cooling induces intracellular ice crystal formation. At slow rates the heat generated by extracellular crystal growth may equal the fall in temperature and will obviate this intracellular nucleation. A third possibility, which explains the occasional intracellular ice, is that at an early stage a single intracellular crystal may arise by a process of seeding through micropores in the cell membrane from the extracellular ice.

Rapid cooling, in which temperatures of the order of −40°C and less can be attained before large extracellular crystals have time to form, is associated with the sudden appearance of very small multiple intracellular crystals by homogeneous nucleation. This is almost invariably fatal except in the case of the small, highly permeable cells.

There can be no doubt that such mechanisms certainly contribute to the death of many cells in a cryo-lesion by the end of a single freeze–thaw cycle, and ice crystals can be demonstrated within the extracellular phase and, occasionally, within the cells of tissues subjected to a cryo-freeze. However, it is difficult to transfer directly the *in vitro* laboratory observations to the *in vivo* situation since the essential measurements such as the temperature and the freezing rates within the cryo-lesion are difficult to assess. The most accurate and reproducible method of

investigation is by means of multiple fixed *in situ* thermocouples, by which method it is possible to demonstrate temperatures and thermal gradients at constant points within the iceball that are not dissimilar to those observed in cell suspension experiments. That is to say, the local changes in no way differ from those associated with a significant survival in cell suspensions, and it must be assumed, therefore, that a proportion of the cells survive the initial thermal injury. This, obviously, is not compatible with the clinical observation that the final picture is of uniform cell death, and investigations seem to confirm that within a few hours of the initial thermal injury there is a second phase, namely, ischaemic infarction. Obliteration of the microcirculation within the frozen volume of tissue occurs, leading to an all or nothing lethal affect. No cells survive the ischaemic infarction regardless of their sensitivity to cold.

There is much circumstantial evidence to support the hypothesis that cellular anoxia is the ultimate lethal mechanism of *in vivo* freezing. It would explain the survival of transplanted cells taken from a cryo-lesion immediately after thawing and the almost complete absence of surviving cells when the tissue is sampled 24 hours after freezing. The uniformity of cell death both at the periphery and the centre of the lesion, despite a steeply decreasing temperature gradient and widely differing freezing and thawing rates, is also supported by this hypothesis. Finally, experiments using both radioactive xenon and intravascular injections of carbon particles tend to confirm that the microcirculation, at first patent after the single freeze, is totally obliterated within one hour or less after the initial insult.

HISTOLOGY

Recognisable morphological changes can be seen immediately after freezing and are easily recognised within 30 minutes after a return to normal ambient temperature. The earliest changes consist of an area of uniformly altered cells sharply demarcated from the surrounding normal tissue, showing pyknotic nuclei, oedematous, coarsely granular and sometimes 'vacuolated' cytoplasm, with slight disruption of the reticulin framework. Immediate fixation of the frozen margin shows the familiar demarcating line, with typical ice crystal spaces in the periphery of the lesion. Initially, there is no clear intermediate zone but within 30 minutes there is a distinct band, between 10 and 30 cells broad, which separates

the injured from the normal area. The cells of this band are more eosinophilic and have coarser cytoplasmic granularity; their nuclei, while not showing the obvious pyknosis seen in the frozen area, are smaller, more basophilic, and have an apparent increase in the number and size of chromatin clumps. The cytoplasmic changes become more marked after two hours but by six hours this intermediate zone is again less distinct. The ultimate fate of this border zone is uncertain but it may be regarded as a zone of transient change in cells which quickly recover. Within one hour the injured tissue commonly shows fragmentation, with a marked increase of the extracellular spaces, much of this space being filled with red blood cells as a result of the vascular stasis and damage to the small vessels. At this stage the cells are shrunken and intracellular detail is obscure. Subsequent changes occur which do not differ greatly from those accompanying an ischaemic infarct. There is a rapidly increasing cellular infiltration composed mainly of polymorphs but also lymphocytes and plasma cells, confined predominantly to the margin of the lesion. There is progressive pyknosis, thrombus formation in vessels, disintegration of the vessel walls, and eosinophilic necrosis. Peripheral fibroblastic activity is seen early, and by the third day there is prominent organisation of the ghost-like cryo-necrotic tissue. A fibrous capsule containing fine capillary-like vessels appears by the fourth and fifth day and surrounds the dead tissue. A gradual absorption of the dead tissue follows, accompanied by progressive inward organisation. Occasional liquefaction necrosis with pseudocyst formation occurs, and eventual collaginisation of the dead tissue produces a small residual scar. Throughout this process the tissues adjacent to the frozen cells show little morphological change and by the time of reorganisation the structures are indistinguishable from normal.

Tissue interphases do not obstruct the freezing boundary and changes such as have been described occur in all tissues. In appropriate sites bone may be incorporated into the expanding iceball and will become devitalised, yet unlike the majority of tissues it does retain its structural integrity. Frozen sections of large blood vessels behave in a similar fashion in that, although devitalised, their walls remain intact as a result of their elastic collagen component. A return of blood flow will occur on thawing in all but the smallest vessels.

Electron microscopy has added little to our knowledge of the effects of very low temperatures other than to confirm the light microscope findings and to allow a more certain identification of small intranuclear ice crystals. It has also confirmed the dramatic demarcation between totally

injured and surviving cells. There are non-specific changes that affect the various cell membranes, and particularly the plasma and nuclear membranes, and it has been suggested that the integrity of the plasma membrane is the limiting factor in cell survival.

Repeated freeze-thaw cycles produce a lesion with histological changes such as nuclear pyknosis and chromatin clumping observed in a single freeze lesion, but in a more exaggerated fashion. After several cycles there is, in addition, progressive loss of the cytoplasmic detail and interruption of cell membrane continuity. Occasional fragmentation of nuclei occurs but the majority remain intact. Despite this 'local homogenisation' there is a striking preservation of cellular detail at electron microscope level.

PHYSICAL PROPERTIES

The growth of a cryo-lesion around a central freezing point, usually the hemispherical tip of a cryoprobe, is associated with local temperatures and freezing rates directly related to the rate of expansion of the lesion through the tissues, that is to say, the rate of advance of the ice water boundary. Ultimately, the cryo-lesion assumes the shape of a crude sphere whose discrete boundary remains equidistant from the tip of the probe after a state of thermodynamic equilibrium is reached between the temperature of the freezing tip and the surrounding tissues. A thermal gradient exists along the radius of this sphere which invariably demonstrates a steep initial rise in temperature in the region of the probe and diminishes as the ice boundary is approached. From the ice boundary through the normal adjacent tissue the temperature gradient again rises steeply, and possibly symmetrically, above the freezing point of the tissue until the ambient temperature of the tissue is reached at a point 6 to 8 mm from the cryo-lesion edge.

The eventual size of the cryo-lesion and its growth are directly related to a number of factors, the most important of which are the probe size, its temperature, and the duration of the freeze. There is a direct relationship between the size of the lesion and the size of the probe and its temperature, while the relationship to the duration of freeze is logarithmic during the period of maximum growth. There may be minor variations in some tissues, but, in general, the dimensions and the characters of the cryo-lesion are constant for a single probe application for a constant temperature and duration. For practical purposes a probe at any given

temperature will produce 80 to 90 per cent of its maximum freezing effect within 15 minutes, even though the absolute maximum effect may require almost two hours of freezing. Successive applications of identical freeze/thaw cycles at the same site will, however, alter this performance significantly. The rate of expansion of the boundary through the tissues is accelerated and the volume of frozen tissue gradually increases with each successive cycle until a new maximum effect is obtained after the fifth to the seventh application. This is greater than the maximum effect that can be produced by a single freeze of unlimited duration using the same probe and temperature, and it appears that this multiple freeze phenomena is a result of the increased thermal conductivity of tissue previously stressed by freezing.

Theoretical consideration would suggest that a number of additional variables related to the target tissue could influence the size and growth of the cryo-lesion. These include the properties of the tissue, collectively termed the thermal diffusivity, the osmolarity of the tissue, and its ambient temperature. In practice, however, with the possible exception of bone, the tissues of the human body are constant in their thermal properties and the effect on the freezing performance is therefore negligible. It is worth remembering, however, that a 5 per cent increase in the dimensions of the iceball can be produced by each 1°C fall in ambient temperature, and for this reason it is possible that a greater volume of tissue may be frozen by pre-cooling of tissues by some means prior to the application of the cryoprobe.

PREDICTION

When surface freezing is employed in clinical practice the extent of cryo-destruction is directly related to the visible colour change, which can readily be observed. If deep-seated tissues are exposed to freezing without direct vision, the control and predictability of the process presents a problem. Mathematical formulae have not provided a satisfactory or a practical means of producing quantitative freezing and the use of thermocouples is essential in this context. Certain requirements are inherent in their use. There must be an appreciation of the temperature changes occurring within and adjacent to the expanding cryo-lesion, and it must be the early depression below the ambient temperature that is detected rather than the edge of the ice ball itself, if over-destruction is to

be avoided. On the other hand, when a growing cryo-lesion involves the shaft of a thermocouple before encroaching on its recording element, cooling of the metal will give a false impression of the existing temperature at that point. If not recognised, this may result in therapeutic under-freezing.

CRYO-IMMUNOLOGY

Recent reports suggest that within a cryo-lesion tumour, destruction releases either tissue proteins, which acquire new antigenic properties, or pre-existing but unavailable antigens. The result is the creation of an autoimmune response to the target tissue that is directly related to the freezing process. This release of antigenic substance probably occurs during the relatively slow thawing period rather than the preceding rapid freeze and, as such, differs from the effect of other forms of tissue injury such as irradiation in which the potential antigenic substance is simultaneously destroyed. The first report of this antigenic response was made by Gonder and Soanes, who demonstrated tissue specific autoantibodies following cryo-coagulation of male rabbit accessory gland of reproduction and it paralleled a clinical report in which metastatic deposits of prostatic carcinoma were seen to regress following repeated cryo-coagulation of the prostatic primary. Since that time, variable antibody responses have been demonstrated after cryosurgery of rectal carcinoma, malignant melanoma, and basal cell carcinoma, a fall in the antiglobulin consumption titre evidently correlating with a decrease in tumour size and subjective clinical impression.

These isolated reports are suggestive of an acquired response against the injured tissue, but it would be inadvisable at this stage to assume that there is firm evidence of a clinically significant immune response against cryosurgically treated tumours. On the other hand, should this phenomenon be substantiated its potential application to cancer therapy would be a most exciting prospect.

CHAPTER 2

CRYOSURGICAL ENGINEERING

Man's capability for heating objects has, until comparatively recently, greatly exceeded his ability to cool them. In many parts of the world snow and ice do not occur naturally, and those areas where large reserves of 'cold' are readily available tend to be both remote and inhospitable. We must accept in principle that nature is not on our side.

Cryogenic engineering, in the practical economic sense, is of recent origin and it was natural that cryosurgery, in its earlier days, should be shackled to certain fixed points on the temperature scale. Ice, solid carbon dioxide or 'snow' and, later, liquid nitrogen were the simplest forms of refrigerant available, and their use in the hands of dedicated pioneers has laid the foundations for a new branch of surgical engineering. At the same time, the very simplicity of these fixed temperature points obscured the underlying physical and biochemical factors involved in tissue destruction. Cryobiologists, who are normally more concerned with the application of low temperature as a means of preserving living cells, are beginning to provide us with a better understanding of these factors which should, in turn, enable better instruments to be designed.

If one accepts the current theories of tissue destruction, then only moderately low temperatures are essential for the formation of an ischaemic infarct, and a further moderate reduction, accompanied by the appropriate rate of cooling and thawing, to satisfy cryobiological concepts.

Animal and human tissues are vascular, and in cooling a given mass of such tissue the dynamic nature of the system must be considered since this will determine the rate at which heat can be abstracted, i.e. the cooling power of the refrigerating mechanism applied. Once the concept of cooling