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Principles of Cutaneous Cryosurgery: An Update

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Cryosurgery · Cryotherapy · Cryobiology · History · Equipment · Techniques · Skin diseases

Introduction

Cryosurgery is the well-aimed and controlled destruction of diseased tissue by application of cold (table 1). It has been shown to be effective and efficient in various skin diseases, provides high cure rates and good cosmetic results with a few contra-indications and low incidence of complications [1].

History of Cryosurgery

The first physician who used freezing to treat a dermatological disease was C. Gerhardt, a German dermatologist from Jena [2]. In 1885, he published a paper on the treatment of cutaneous tuberculosis with cold. Gerhardt had built a system, in which the lesions were covered with ice bladders for 3 h twice a day. Four patients considerably improved after a 2- to 4-week treatment. In 1899, A.C. White, an American dermatologist from New York, used for the first time liquefied air to treat various skin disorders, such as verrucae vulgares, naevi, precancerous lesions and tumours. In the year 1905, M. Juliusberg, a dermatologist from Berlin, introduced the term 'cryotherapy' for the treatment of skin lesions with cold. He applied the first cryospray, a small balloon filled with carbon dioxide released in spurts.

Table 1. Terminology of using subzero temperatures in biology and medicine

Cryobiology	Effects of subzero temperatures on a living system
Cryogenics	Development of freezing temperatures within a living tissue or cell
Cryotherapy	Therapeutic use of cold in a wide sense
Cryosurgery	Well-aimed and controlled destruction of diseased tissue by application of cold

Modern cryosurgery was born in the 1960s after liquid nitrogen became available, and closed-circuit devices working with liquid nitrogen, freon gas and nitrogen protoxide have been developed by the American neurosurgeons I.S. Cooper and A.S. Lee and their Italian colleagues V.A. Fasano, G. Broggi, T. de Nunno and P. Baggiore. Nowadays, numerous sophisticated devices have been developed and commercialized, not only to preserve and deliver cryogens, but also to monitor temperatures in and underneath the treated lesions, thus allowing a controlled and reliable cryosurgery of the diseased skin.

Cryobiology

The biological changes that occur during and after cryosurgery have been studied in vitro and in vivo and are caused by reduction of tissue temperature and consequent freezing [3–6]. Tissue injury is induced by cell freezing and by the vascular stasis that develops in the tissue after thawing. The cryoreaction is, therefore, characterized by the

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Table 2. Factors affecting the effects of freezing in tissue (from Zouboulis [1]): Optimum parameters for the treatment of skin diseases

	Benign lesions	Malignant lesions
Speed of tissue freezing	Moderate or rapid speed (≤100 or >100 °C/min)	Rapid speed (>100 °C/min)
Speed of thawing	Slow speed (10 °C/min or spontaneous rewarming)	Slow speed (10 °C/min or spontaneous rewarming)
Intra-/extracellular osmotic phenomena	Heterogenous and/or homogenous nucleation	Homogenous nucleation
Probe tip temperature	–86 or –196 °C	–196 °C
Tissue temperature	–20 to –25 °C	–50 °C or lower
Duration of freezing	30 s	30–60 s
Repetition of freeze-thaw cycles	No	Yes (twice)
Vascular reaction	Yes	Yes
Immunological reaction	Probable	Probable

physical and the vascular phases. A postulated third phase of cryoreaction, the immunological phase, is not well studied in the skin. The factors affecting the effects of freezing on tissue and their optimal parameters for the treatment of skin diseases have currently been reported [1] (table 2).

Physical Phase of Cryoreaction

Tissue Freezing: Homogeneous and Heterogenous Nucleation. Rapid to very rapid freezing (100–260 °C/min) leads to cellular death [4, 7]. Intracellular ice formation damages cell organelles, such as mitochondria and endoplasmic reticulum, inducing an irreversible cell destruction known as *homogenous nucleation.* Ice crystal size is important, since the larger the crystals are, the greater damage they induce. Rapid freezing speeds are required in the treatment of malignant skin tumours where cryosurgery has to be lethal. In the treatment of benign skin lesions, moderate freezing speeds (up to 100 °C/min) lead to differential freezing of the tissue compartments consequently resulting in extracellular ice formation, hypertonic and sensitization damage. These phenomena which can also induce an irreversible cell destruction are known as *heterogenous nucleation.* Extracellular ice formation alone is not sufficient to kill cells since disruption of cell membranes barely occurs despite the volume changes in the extra- and intracellular compartments. When extracellular ice is formed, changing osmotic gradients between cells and extracellular fluid are produced which lead to a passage of electrolytes out of the cells giving a decrease in cell volume. At reaching a certain concentration of essential intracellular molecules, they also abandon the cell causing irreversible cell damage (hypertonic damage). However, gross cell damage can be observed even when the necessary hypertonic conditions are not achieved. This leads to the assumption that phospholipid disruption in cell membranes after cryosurgery may lead to cell death.

Slow freezing speeds only induce extracellular ice formation and together with the addition of cryoprotective agents, such as dimethyl sulphoxide, which prevent hypertonic damage, they are used in cryoconservation of cells and tissues.

Tissue Thawing. A slow thawing speed (10 °C/min) induces volume changes in the extra- and intracellular compartments leading to an increase in the intracellular water content. Rapid electrolyte transfer has been incriminated as the cause of damage to cell proteins and enzyme systems. Reverse osmotic gradients during thawing may give rise to cell damage. In addition, intracellular recrystallization of water is also responsible for tissue destruction. The latter process is as important as the initial freezing in causing cell death. Adequate freezing has been performed when the thawing time is 1.5 times the freezing time or longer.

Tissue Temperature. Freezing takes place in the tissue at –0.6 °C, but this is not the lethal temperature. Various skin cell populations present a distinct ability to tolerate cold [8–10]. Melanocytes are the skin cells most sensitive to low temperatures, they die at –4 to –7 °C. Cells of the sebaceous glands and hair follicles are also rather sensitive to cold; already temperatures lower than –20 °C are lethal for them. Keratinocytes die at about –20 to –30 °C, while fibroblasts are rather resistant to cold, they die at –30 to –35 °C. It is, therefore, difficult to achieve optimum cooling rates capable of killing all skin cells during cryosurgery. Theoretically, formation of ice crystals in tissue and, therefore, tissue freezing, starts from temperatures lower that –21.8 °C, which is the eutectic temperature of sodium chloride solution [4]. In rapidly dividing cells, the water content is directly proportional to the mitotic index, hence these cells

are more likely to be damaged. A probe tip temperature lower than –180 °C and a tissue temperature at least as low as –50 to –60 °C have been shown essential to kill all target cells [10–12]. These parameters are required in cryosurgery of malignant skin tumours; an optimal cryosurgery of benign skin lesions only requires tissue temperatures of –20 to –25 °C.

Duration of Freezing. Cell death rates have been shown in vitro to increase not only with lower temperatures but also with longer freezing times [3]. The effect of freezing on cell viability reaches a maximum at about 100 s followed by a plateau in cell death rates with time.

Repetition of Freeze-Thaw Cycles. The importance of more than one freeze-thaw cycle in causing increased rates of cell death has been demonstrated in several in vitro and animal studies [3, 13]. Electron-microscopic studies of normal skin showed damage to all cell structures after a second freeze-thaw cycle [14]. Repeated freezing-thawing cycles are essential in the treatment of cutaneous tumours [14, 15] but are not required in the treatment of benign skin lesions [16, 17].

Vascular Phase of Cryoreaction

Cryogenic injury leads to vascular stasis and tissue anoxaemia resulting in ischaemic necrosis [7, 18]. Microscopic examination of injured tissue in animals has shown that oedema, focal capillary damage, haemorrhage and isolated microthrombi begin to occur after 2 h and that by 5–8 h focal or segmental necrosis of blood vessels is present. Gangrene clinically appears between 1 and 7 days, but only when injury is severe. Even after mild cold injuries, the initial circulatory impairment is irreversible, thus implicating delayed progressive thrombosis as the main factor producing tissue loss [19, 20]. Thrombosis in 65% of the capillaries and 35–40% of the arterioles and venules already develops at tissue temperatures of 11 to 3 °C, while thrombosis of all vessels is detectable at –15 to –20 °C [18].

In an attempt to explain exudation which occurs after cryosurgery, ultrastructural studies of endothelial cells have shown that cell damage in the first hour after freezing and thawing includes rupture of cell membranes, thinning and later condensation of ground substance and swelling of rough endoplasmic reticulum and mitochondria [21].

Immunological Phase of Cryoreaction

An immunological response after cryosurgical treatment [17] was first suggested in the late 60s/early 70s when circulating antibodies were observed directed against prostatic or adrenal tissue following freezing in both rabbits and humans. Clinically, regression of tumour masses beyond the region treated by cryosurgery or even in distant metastases has been observed. However, antibody response seems to be unspecific, since it is directed against tissue antigens rather than tumour ones. Currently, confirmation of an immunological response to cryosurgery has been provided. Natural killer cell activity was found enhanced following cryosurgery of normal liver and liver tumours in animals and in humans. On the other hand, cryosurgery induced alterations in membrane proteins of cell ascites fibrosarcoma cells in mice and immunological response after treatment of liver carcinoma in rats. There are still only a few studies on the immunological cutaneous response to cryosurgery. Langerhans cell activity was found enhanced in mouse skin after treatment [22]. In cryosurgery of human keloids, tenascin expression, which was initially restricted to the diseased tissue, became diffuse in the whole treated dermal region and consequently decreased, while IFN-γ expression was depleted after cryosurgery [23].

Cryosurgical Equipment and Treatment Techniques

Simple Cryosurgical Units: The Cotton-Tipped Applicator and the 'Hard-Tail' Dip-Stick

The simplest cryosurgical modality, still in current use, is the cotton-tipped applicator method which applies small or large swabs soaked in liquid nitrogen [17]. Both instruments lack the capacity of active freezing and, therefore, can only provide a slow freezing speed. Large swabs create large frozen surfaces that may override the margins of the area intended to be treated, while small swabs have a limited liquid nitrogen reservoir capacity. An improvement of the classic cotton-tipped applicator is the 'hard-tail' dip-stick [24]. It is made out of a standard large cotton-tipped applicator with a pointed end ('tail') which does not exceed 5 mm. A large amount of liquid nitrogen is absorbed by the cotton reservoir and is slowly released at the tip of the swab, producing an accurate freezing effect. The method is only sufficient to treat epithelial benign lesions, especially common warts. To avoid cross-contamination, a used swab is never to be soaked again in the liquid nitrogen supply, but a sterilizable metal device to hold a small portion of liquid nitrogen sufficient for treatment of a single patient can be used [25].

The Modern Cryosurgical Unit

Nowadays, there are many commercially available, well-functioning cryosurgical units with variable design, function and performance characteristics [26, 27]. Sufficient cold for

cryosurgery can be produced by direct or indirect application of a solid or liquid cryogen stored at low temperatures, by lowering the pressure of a gas (Joule-Thompson effect), eletromechanically or simply by refrigeration. The devices are mainly characterized by the applied cryogen and the manner of cryogen application to the skin [1]. A cryosurgical unit consists of five main components: a liquid Dewar/gas cylinder, the cryogen, a pressure gauge, a cryogun with tubing and assorted cryoprobe/spray tips.

The Liquid Dewar/Gas Cylinder. Liquid Dewar and gas cylinders are of widely varying size. The efficiency of cryosurgery using nitrous oxide is dependent on maintaining adequate gas pressure within the cylinder. Internal gas pressure decreases during freezing and must regenerate at room temperature between freezes. In contrast, most units using liquid nitrogen are not pressurized until the unit becomes activated. They incorporate regulators to reduce or control tip pressures for economy and safety. The regulators provide a constant performance at various cylinder pressure levels.

The Cryogen. The cryogen mostly used nowadays is liquid nitrogen which can generate low target tissue temperatures because of its boiling point of –195.8 °C. It is considered to be the cryogen of choice for cutaneous cryosurgery and the only cryogen advocated for treatment of malignant skin lesions. Nitrous oxide, a non-flammable gas with a boiling point of –89.5 °C, is a sufficient cryogen for the treatment of benign skin lesions.

The Gas Presssure Gauge. Certain cryosurgical units feature a gas pressure gauge located between the cylinder and the cryogun. The gauge indicates the pressure within the cylinder and is divided into three pressure zones. The high zone of the gauge reflects excessive cylinder pressure (a safety hazard), and the low gas pressure results in inefficient and probably inadequate freezing. The middle zone indicates adequate freezing.

The Cryogun. The cryogun consists of a hand grip, activation trigger, cryogun stem and the cryoprobe stem. In some units, the on/off switch for the gas valve is located on the cryogun, in others it consists of a pedal. In the majority of the units, depression of the activation trigger initiates the freeze, in a few others it defrosts the probe tip. Some triggers feature a locked position setting so that the trigger needs not to be depressed during the freeze.

Cryoprobe and Spray Tips. Cryoprobe tips must be made of a good thermal conducting metal, such as silver, gold or copper. The interchangeable tips are available in various shapes and sizes to enable maximum contact of the tip with the tissue and avoid freezing of heatlhy areas. The majority of cryosurgical units feature pyrometers, which indicate the actual temperature of the cryoprobe tip. By applying the spray technique, spray tips with different aperture sizes are used. Intralesional cryosurgery engages single-use, 20-gauge or larger needles instead of tips (fig. 1).

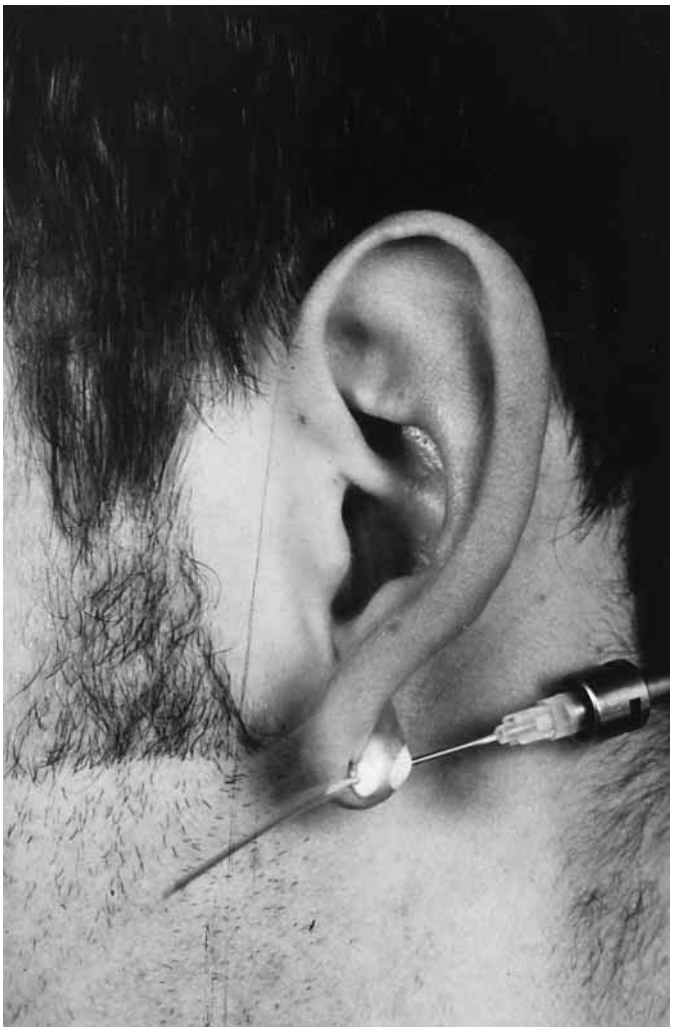


Fig. 1. Intralesional cryosurgery: liquid nitrogen is passed through the lumen of a needle exiting to the atmosphere from its end. An ice cylinder is formed around the embedded part of the needle within the tissue. The needle is connected to a small liquid nitrogen Dewar device by a flexible, long metallic cryoprobe stem.

Classification of Cryosurgical Devices According to the Cryogen Used

Liquid Nitrogen Units. Liquid nitrogen units are open systems that can be used for both cryoprobe and spray applications. They develop a probe tip temperature of –170 to –196 °C and, therefore, a rapid freezing speed of over 100 °C/min. Hand-held simple units with a liquid Dewar capacity of 250 ml to 1 litre as well as large instruments

Table 3. Advantages and disadvantages of high-pressure and liquid nitrogen devices

High-pressure devices (nitrous oxide)	Liquid nitrogen devices
Advantages	
Relatively inexpensive and robust	Freezing at rapid speed
The working fluid is supplied at room temperature, highly insulated supply lines are not required	Very low temperature at the cryoprobe tip
They operate efficiently without precooling; the cooling expansion occurs within the tip itself	Available for both contact and spray techniques
Disadvantages	
Freezing at moderate speed only	More expensive due to the quick evaporation of liquid nitrogen and the requirement of a storage Dewar flask
Moderately low temperature at the cryoprobe tip	Insulated supply lines are required
Available for contact technique only	The cooling expansion occurs within the whole device

with sophisticated temperature controls and a liquid nitrogen Dewar capacity of up to 10 litres are available. Most units are not pressurized until treatment starts. Liquid nitrogen requires a storage container.

Nitrous Oxide Units. Nitrous oxide units are closed systems which operate using the Joule-Thomson effect. They can be practically only used for cryoprobe applications. This method of refrigeration results from the expansion of the gas through a small opening (adiabatic principle). Pressurized nitrous oxide advances down the narrow cryogun stem (high-pressure cryoprobe). When it reaches the hollow cryoprobe tip, the gas rapidly expands, lowering it to a temperature below freezing. The temperature of the nitrous oxide cryoprobe drops to –65 to –86 °C and, therefore, a moderate freezing speed of less than 100 °C/min is developed. High-pressure devices have several advantages over liquid nitrogen probes (table 3).

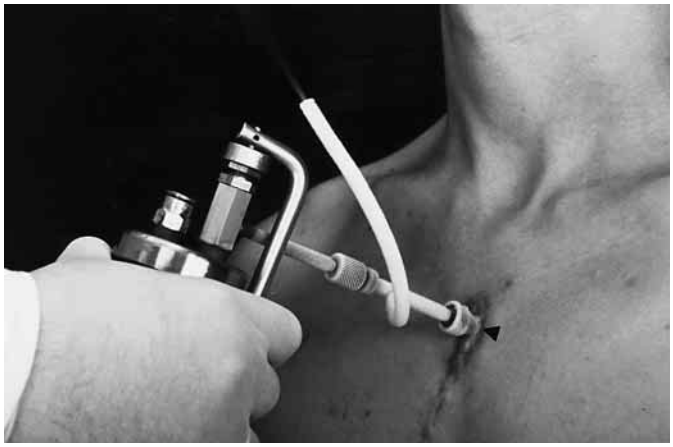
Units Using a Peltier Thermo-Electric Element. Units using a Peltier effect cooler are closed systems only used for cryoprobe applications. They develop a probe tip temperature of –32 to –42 °C by a thermo-electric procedure, and, therefore, they do not need a cryogen. The low freezing speed makes these devices only sufficient for the treatment of superficial benign epithelial and pigmented lesions (solar lentigines) [28] and in cosmetic dermatology.

Classification of Techniques According to the Method of Cryogen Application

The methodology of cutaneous cryosurgery has nowadays been sophisticated and the techniques standardized. There are three different techniques to be used for the treatment of skin lesions.

The Contact Technique. The contact method uses metallic probes which function after the principle of temperature exchange (fig. 2). These probes are circulated by a gas cryogen. As the tip removes heat from the tissue, the tissue gradually cools. The size, material, composition and temperature of the probe tip determine its tissue-cooling capacity. Other factors, such as tissue moistness, extent of tissue contact, the duration of freeze and pressure exerted on the probe, affect heat diffusion. When the cryosurgical unit is activated and the probe is placed in firm contact with the tissue, an area of frozen tissue or 'iceball' may be observed extending radially from the cryoprobe tip. The interface between the iceball and unfrozen tissue represents the 0 °C isotherm, which is the line of connection points representing 0 °C at the given time. The longer the duration of freeze, the further the iceball radiates from the cryoprobe tip margin [3]. The distance between the tip margin and the 0 °C isotherm represents the lateral spread of freeze. The depth of the 0 °C isotherm from the tip indicates the depth of freeze. Although variable, the lateral spread of freeze approximates the depth of freeze by a ratio of 1:1.3 [29]. The volume of tissue located between the –22 °C isotherm and the probe tip is called the lethal zone. Cells within this zone undergo cryonecrosis [4]. Those cells located in the warmer region between the –22 °C isotherm and the 0 °C isotherm generally survive the freeze. This important area represents the recovery zone. The contact method provides controllable as well as reproducible results. The results can be modulated by pressing the cryoprobe against the skin, thus inducing vessel contraction. A maximum depth of freezing of 20 mm can be achieved [30].

The Spray Technique. The spray technique uses an open freezing system with freeze secure vents (fig. 3). It directly emits a fine spray of a cryogen at the target area. It is particularly useful for irregular lesions and lesions with a curved surface. For large lesions to be treated in one session, a paint brush or spiral pattern of spray can be used [31]. The spray



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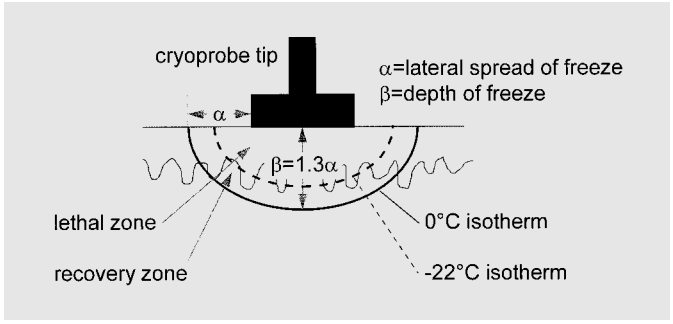

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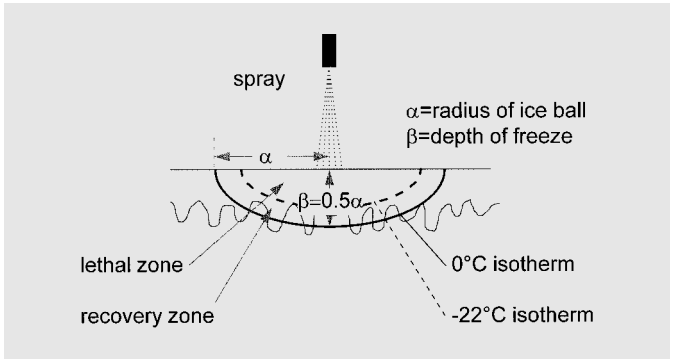

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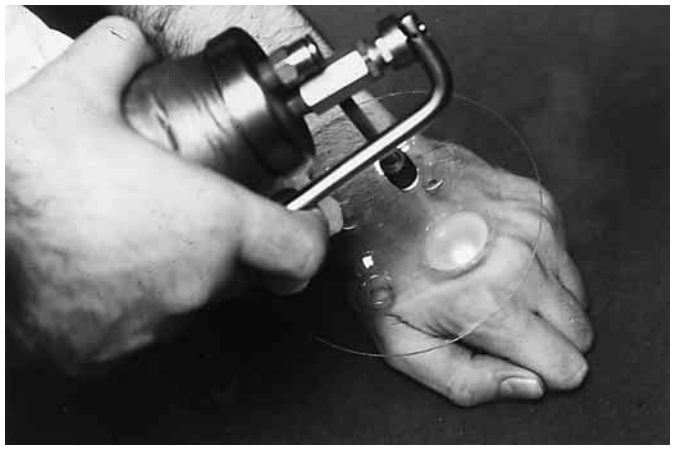

b

is emitted from a distance of 1–2 cm from the target site and at a 90-degree angle to it. The depth of the freeze may be judged by the lateral spread of freezing on the surface; it is about half the radius of the surface area [32]. An intermittent spraying of liquid nitrogen is desirable since it results in a more uniform temperature in the iceball and greater depth, while it limits lateral spread. The depth of freeze can only reach 10 mm and, therefore, it is not the appropriate technique for voluminous lesions. To increase efficiency, curettage of the exophytic part of the lesion is often performed [30]. There are two variants of the spraying procedure, the described open-spray technique and the confined-spray technique. The latter directs the spray into cones [27], individually prepared plastic moulages or other materials which are open at both ends and one end is placed on the skin. The confined-spray technique restricts the spray to the lesion and avoids wide freezing of the healthy peripheral tissue; however, it makes impossible the clinical evaluation of the depth of freeze.

Intralesional Cryosurgery. The disability of skin surface cryosurgery to freeze deeper than 20 mm has led Weshahy [33] to develop a method for applying cryosurgery in depth. One or more needles are introduced into the skin from one point, run through the deeper part of the lesion and appear at the surface on the opposite border. A sprayed cryogen is then passed through the needle by inserting the spray tip of the cryosurgical device into the head piece of the needle. The cryogen is passed through the lumen exiting to the atmosphere from the other end of the needle. An ice cylinder is formed around the embedded part of the needle within the tissue. The distance of extension of freezing can be clinically estimated by the degree of extension of the whitish iceballs formed around the points of contact between the skin surface and the visible portions of the needle. The needles can be angled, curved and hook-shaped. Compression of the lesions is accomplished by pulling up the visible parts of the needle. Modifying the Weshahy technique, we have developed a device constituted of a small liquid nitrogen Dewar device engaging a single-use, 20-gauge needle to spray liquid nitrogen which is connected to the device by a flexible, long metallic cryoprobe stem (fig. 1). The cryo-

Fig. 2. a Contact freezing technique: when the cryosurgical unit is activated and the probe is placed in firm contact with the tissue, an area of frozen tissue or 'iceball' may be observed extending radially from the cryoprobe tip (arrowhead). **b** Iceball induced by a cryoprobe tip.
Fig. 3. a Iceball induced by a cryogen spray. **b** The confined-spray technique: the liquid nitrogen spray is directed into a plastic moulage with an opening size fitting the size of the lesion to be treated.

probe stem is Luer-locked to the needle. The shape of the cryoprobe stem is variable so that the Dewar cylinder can stay upright during freezing. The shape of the needle can also be changed in order to form a hook. Main advantages of intralesional cryosurgery could be the freezing of the entire lesion independently from lesional volume and the minimum surface destruction compared to the contact and spray techniques which can be further reduced by using peripherally insulated needles.

Methods of Monitoring Tissue Temperature

The depth of tissue to be frozen should be exactly monitored in cryosurgery of malignant skin tumours [27]. In the treatment of benign skin lesions monitoring of freeze depth is optional. The progress of freezing can be clinically judged by the duration of freezing, the thawing of the lesion and the measurement of the lateral spread of freeze (contact technique) or the radius of the iceball. To supplement clinical estimation, the tissue temperature can be monitored with a pyrometer-thermocouple apparatus using the thermocouples mounted in 25- to 30-gauge needles. The thermocouples are inserted into the skin so that the tip lies beneath or lateral to the lesion. Other monitoring techniques are the measurement of the electrical impedance in frozen tissue and ultrasound [34, 35]. Further improvement could provide a device combining a nitrous oxide cryoprobe and an ultrasound microtransducer (20 MHz) at the centre of the probe tip permitting ultrasonographic monitoring of the tissue during freezing [36].

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