In Vivo Results with a New Device for Ultrasonic Monitoring of Pig Skin Cryosurgery: The Echographic Cryoprobe

Pascal Laugier,* Emmanuel Laplace,*† Jean-Louis Lefaix,‡ and Geneviève Berger*

*Laboratoire d’Imagerie Paramétrique, URA CNRS, Paris, France; †DATE, La Motte d’Aveillans, France; ‡Laboratoire de Radiobiologie Appliquée, CEA, Gif-sur-Yvette, France

One of the main difficulties encountered in cryosurgery is the uncertainty in the extent and depth of the tissue effectively treated during the freezing process. The objective of this study was to evaluate in vivo ultrasonic control of skin cryosurgery using a new echographic cryoprobe. An echographic cryoprobe, developed specifically for dermatology applications, combines a high-frequency (20 MHz) miniature ultrasonic transducer and a N₂O-driven closed cryoprobe. Knowledge of the ultrasound velocity of frozen skin is a prerequisite for monitoring the iceball formation kinetics. Therefore, in a first study, we estimated the ultrasound velocity of frozen skin specimens. In a second step, the operation of the echographic cryoprobe was assessed, under in vivo conditions similar to those used in human therapeutics, on normal skin of three female “Large-White” pigs under anesthesia. The mean value of ultrasound velocity of frozen skin obtained by pooling the data from all the skin specimens included in this study was 2865 ± 170 m per s. The average rates of growth (10⁻² mm per s) of the iceballs were found to be 12.2 ± 1.0 (pig 1), 9.0 ± 1.0 (pig 2), and 8.4 ± 0.9 (pig 3). The echographic cryoprobe had a built-in high-frequency ultrasonic transducer that served two functions. It enabled in vivo real-time monitoring of depth penetration of the iceball and it gave important feedback to the operator or to the console relating to the rate of growth of the iceball. Automatic (i.e., operator-independent) detection of the echo signal from the freezing front and calculation of the depth penetration of the iceball was possible. Key word: ultrasound. J Invest Dermatol 111:314–319, 1998

Cryosurgery is the use of subzero temperatures to destroy abnormal tissues. A coolant or cryogen is circulated through a cryoprobe that is placed in contact with the tissue to be destroyed. The extraction of heat from the tissue at the cryoprobe tip produces a frozen region that penetrates into the tissue, forming a hemispheric freezing interface. Cryosurgery has become a well-established therapy in dermatology and treatment success rates are often over 95% (Graham, 1993).

A precise control of the iceball growth during treatment is necessary to protect healthy tissues, vital organs, and major blood vessels. Several methods are currently applied to control depth and extent of tissue insult. For skin tumors this is usually accomplished using clinical observation, palpation, surface extent of the frozen region, or timing of the freeze. These methods are not applicable for the treatment of deep lesions in other parts of the body.

Several techniques for monitoring the freezing process have been developed. Thermocouples, inserted in the tissue at the boundary of the lesion, are in use to measure the depth of destructive temperature. An alternative method, using low-frequency alternating current transmitted between needle electrodes, measures the change in the electrical impedance of the tissue due to freezing (Le Pivert et al, 1977; Gage, 1979; Price and Biro, 1983). Most crucial is the accurate placement of thermocouples or electrodes required for a precise control of the treatment. A high accuracy is rarely achieved when these methods are used in vivo, unless ultrasound is used to guide needle insertion (Abramovits et al, 1996).

Imaging technologies, including magnetic resonance imaging, X-ray computerized tomography, and ultrasonic imaging, are evaluated as tools to monitor the freezing process of tumors in internal organs. Whereas magnetic resonance imaging and X-ray computerized tomography have been used for monitoring cryosurgery of liver, brain, and kidney (Matsumoto et al, 1992, 1993; Gilbert et al, 1993; Reiser et al, 1993), and have shown considerable promise, both techniques are expensive and require substantial equipment. Moreover they do not provide real-time imaging.

The dramatic improvement in imaging has been provided predominantly by ultrasonography. Real-time ultrasound imaging with widely available, portable, radiation-free, and easy-to-use high-resolution echographic equipment has been shown to be able to monitor the freezing process to ensure that the entire lesion was encompassed by the freezing edge while sparing uninvolved tissues, so that cancerous tissue was optimally killed by the freezing process. Improved ultrasound monitoring of the frozen volume has led to the reawakening of interest in cryosurgery in several parts of the body, mainly prostate and liver (Tanahashi et al, 1977; Onik et al 1985, 1991, 1993a; Quigley et al, 1992; Littrup et al, 1994; Pittrof et al, 1995; Polk et al, 1995; Uchida et al, 1995; Wieder et al, 1995). In these studies, commercial ultrasound clinical scanners with probe frequencies ranging from 3.5 to 7.5 MHz were used. Intraoperative ultrasound for the liver and transrectal probes for the prostate were used for monitoring tissue freezing, and showed an echogenic rim with posterior acoustic shadowing corresponding to the iceball formation around the cryoprobe tip. Cryosurgery of prostate or liver has been suggested and used as an alternative in patients who could not undergo surgical resection. Several studies using ultrasound monitoring of cryosurgery have found that long-term survival rates

0022-202X/98/$10.50 · Copyright © 1998 by The Society for Investigative Dermatology, Inc.
could be achieved in patients with previously unresectable hepatic tumors (Onik et al., 1993b; Shafir et al., 1996).

We have previously demonstrated on excised skin specimens that high-frequency ultrasound (15 MHz) could be used to detect the depth boundary of the frozen region (Laugier and Berger, 1993). Despite the encouraging results obtained on organs such as prostate or liver, similar coupling of the cryosurgical process and real-time ultrasonic monitoring for dermatology was difficult under in vivo conditions because of the superficial situation of skin lesions: the ultrasonic examination of the frozen site could only be achieved after removal of the cryoprobe.

In order to generalize real-time ultrasonic monitoring to skin cryosurgery, we have developed a new echographic cryoprobe combining the cryoprobe and the ultrasound transducer. To be optimally clinically useful, the device should provide at least two-dimensional B-scans permitting a cross-sectional view of the growing iceball. In a first stage of development, however, a prototype was designed to follow the advancing freezing front along a fixed single direction (i.e., along the ultrasonic transducer axis). This echographic cryoprobe has been tested in vivo on pig skin. The device is limited by the fact that it supplies the position of the freezing front in the direction of a single ultrasonic A-scan. Its extension to echographic monitoring of skin cryosurgery using B-scans is currently underway.

**BASIC PRINCIPLES**

First, we present here a brief summary of the basic principle of pulse-echo measurements. The ultrasonic pulse wave, launched by the transducer in response to an electric excitation, travels through the propagation medium characterized by its acoustic impedance $Z = \rho c$, where $\rho$ is the bulk density and $c$ the ultrasound velocity, ranging from 1450 m per s to 1700 m per s in soft biologic tissues. When the ultrasonic wave encounters an interface between two different media (i.e., with different acoustic impedances), the incident energy is partly reflected back in the direction of the ultrasonic pulse, and partly transmitted through the interface. The distance $z$ between the transducer and the echo–producing interface is estimated by identifying the corresponding echo on the echogram and measuring its time of flight given by $t = 2z/c$.

Experimental aspects of ultrasonic monitoring of cryosurgery have been previously reported (Gilbert et al., 1984; Onik et al. 1985; Laugier and Berger, 1993). Ultrasound pulses are reflected at the boundary of the frozen region, namely the “iceball” surrounding the cryoprobe tip. Ultrasound reflection is caused by the acoustic impedance mismatch induced by the dramatic variations of density and ultrasound velocity encountered at the interface between frozen and unfrozen tissues. Tissue freezing can be monitored ultrasonically in real time as it extends out from the cryoprobe as an echogenic rim. It has been shown that the sonographic appearance of the section of frozen tissue or “iceball” correlated almost exactly with the amount of frozen tissue (Gilbert et al., 1984).

The echographic cryoprobe, designed for skin cryosurgery, combined the ultrasonic transducer with the cryoprobe (Fig. 1). A prototype has been constructed using a modified dermatology cryoprobe (DATE, La Motte d’Aveillans, France) of 23 mm in diameter, supplied with nitrous oxide (N2O Joule–Thomson effect). A hole of 5 mm in diameter was drilled in the center of the cryoprobe for the ultrasonic transducer housing. For this reason, the intake of nitrous oxide that is usually centered on standard cryoprobes currently used in dermatology, was shifted laterally. In order to ensure a homogeneous distribution of the cryogen inside the cryoprobe tip, a circular capillary tube delivered the nitrous oxide at three different equidistant points. These modifications did not alter the performance of the cryoprobe (Laplace, 1997). A miniature high frequency transducer was inserted in the central hole and maintained in place by a small inner stop ring of 0.5 mm thickness at the bottom of the cryoprobe tip.

The expansion chamber of the cryoprobe was cooled to −89°C by Joule–Thomson expansion of the nitrous oxide, thus bringing the external wall of the cryoprobe tip to about −40°C. When the echographic cryoprobe was placed on the skin surface, it produced a fast cooling effect and a growing iceball from the cryoprobe surface. During the freezing process, the ultrasonic measurement consisted of measuring the time of flight of periodically launched pulses, propagating through the layer of frozen skin, and reflected by the iceball boundary. The size of the growing iceball can be derived from the equation $t = 2z/c$, under the condition that the ultrasound velocity in frozen skin was known. Therefore, in a first study, we estimated the ultrasound velocity of frozen skin. In a second step, we evaluated the operation of the echographic cryoprobe for dermatologic use, under in vivo conditions similar to those used in human therapeutics.

**MATERIAL AND METHODS**

**Ultrasound velocity of frozen skin** The velocity of frozen skin was measured using the method described previously (Laugier and Berger, 1993). For the sake of clarity, the main steps of the method are briefly outlined here.

The skin specimens were frozen under conditions similar to those used in human skin cryosurgery. We used a high pressure N2O-driven cryoprobe of 20 mm in diameter (DATE, La Motte d’Aveillans, France), currently used in dermatology. Ultrasonic measurements were performed with a high-frequency focused transducer (Panametrics, Waltham, MA) of center frequency 20 MHz (13 mm in diameter, focal distance of 19 mm). The high-frequency probe provided an axial resolution of about 100 µm, which was adequate for ultrasonic skin investigation and freezing monitoring. The transducer was driven by a pulser-receiver Panametrics 5052 PRX75 (Panametrics, Waltham, MA). The echographic radiofrequency signal was amplified, filtered, digitized at a sampling rate of 400 MHz with an 8-bit dynamic range digital oscilloscope (LeCroy 9450 A, Chestnut Ridge, NY), and stored for off-line processing.

The measurements were performed on five skin samples from domestic pig. The experimental set-up is depicted in Fig. 2. The transducer was immersed in a normal saline bath, at 37°C, controlled in temperature. Fresh circular pig skin specimens of 5 cm diameter and 4 mm thickness were partially immersed in the bath and placed horizontally at the water surface. The outer surface of the epidermis was exposed to the air and the cryoprobe tip was gently pressed against the central part of the epidermis surface. In contrast to the experimental arrangement in the echographic cryoprobe, here the transducer and the cryoprobe were positioned on opposite sides of the skin specimens, so that the growing freezing front moved away from the cryoprobe and towards the transducer. The skin specimens were located in the focal zone of the transducer. Echographic data were recorded every second during a period of 100 s from...
The principle of velocity measurements in frozen skin is illustrated in Figure 4. A cryoprobe echo was generated. The time of flight $t_2$ of the echo of the cryoprobe during freezing was different from $t_0$ because of the velocity variations in the frozen tissue. The time of flight $t_0$ of the cryoprobe echo before freezing was compared with the time of flight $t_0$ due to the nonuniform temperature distribution in the tissue, the ultrasound velocity is a complex function of the spatial coordinates; however, for simplicity, temperature gradients were neglected and we considered, in the first approximation, that the partially frozen skin specimen consisted of only two different regions: a frozen region with constant velocity $c_f$ and an unfrozen region with constant velocity $c_u$. The velocity in the frozen tissue could then be derived using the following equation (Laugier and Berger, 1993):

$$c_f = c_u - \frac{t_0 - t_1}{t_2 - t_1} \quad (1)$$

Only the differences in times of flight were required. The time intervals $t_0 - t_1$ and $t_2 - t_1$ were determined by detection of the points of maximum amplitude on the corresponding waveforms. The value of velocity $c_u$ of normal pig skin was derived from previously reported measurements in the literature ($c_u = 1720 \text{ m per s}$; Goans et al, 1977; Cantrell et al, 1978). A value of ultrasound velocity of the frozen skin was derived from each echographic line recorded during the freezing cycle. Mean values and SD of $c_f$ are reported for each specimen and each cycle.

**In vivo monitoring of cryosurgery** Ultrasonic data were acquired during freezing cycles on three pigs (weighted $\approx 120 \text{ kg}$) under anesthesia. Experiments were carried out in the Laboratory of Applied Radiobiology (CEA, Gif-sur-Yvette, France). All the pigs were female; Large-White, and were provided by the collaborating laboratory (Authorization n°5564 of the Department of Agriculture, Forestry, and Fishing). Freezing was performed on several sites of the outer thigh of the pig skin (five different sites for pig 1, and six sites for pigs 2 and 3). These sites were preliminarily shaved. Pigs were anesthetized with an IM injection of Imalgene 1000 (ketamine: 4 ml) and placed under gaseous anesthesia (Halothan 2%, 66% oxygen, and 33% nitrous oxide).

The echographic cryoprobe has already been described above. It was used in combination with a high-frequency transducer. Because of the small thickness of tissue investigated (a few millimeters), a 20 MHz broadband transducer was selected. The characteristics of the transducer (Physical Acoustic, Princeton, NJ) were as follows: diameter of the active element, 3.6 mm; external diameter, 5 mm; center frequency, 20 MHz; –20 dB frequency bandwidth, 35 MHz; axial resolution, about 75 µm. Acquisition of ultrasonic data was performed using a DPR35 (JSR Ultrasonics, Pittford, NJ) pulser-receiver. The echographic signal was then digitized at a sampling rate of 100 MHz by an 8-bit dynamic range board SFT4100 (Sofrastet, Ecquevilly, France) and transferred to a PC computer for processing.

The echographic cryoprobe was coated with a contact gel. In response to the delivery of the cryogen in the cryoprobe, an iceball penetrated the tissue layer in contact with the cryoprobe surface and thickened gradually until the device was put in the thaw mode. To follow the kinetics of the iceball formation (i.e., size and rate of growth), a total of 200 echographic lines were recorded at a rate of one line per s during a freeze/thaw cycle. The freezing period lasted 110 s. Real-time estimate of the size of the growing iceball could be derived from each recorded echographic signal. The echo signal from the iceball wall was first identified (Fig 5), the time of flight of the echo signal $t_2$ was measured by detection of the point of maximum amplitude. Then, applying the equation:

$$\text{Thickness} = \frac{c_f t_2}{2} \quad (2)$$

where $c_f$ is the average value of ultrasound velocity of frozen skin previously determined, provided an estimate of the thickness of the ice layer.

The estimation of the average rate of growth of the iceball in the dermis (in mm per s) for each freeze site and each animal included several steps: (i) selection of one echographic line recorded shortly after initiation of freeze; (ii) selection of one echographic line recorded when the iceball wall had reached the dermis–hypodermis interface; (iii) identification of the echo from the iceball wall on the two echographic lines; (iv) measurement of the time of flight of these echo signals by detection of the maximum echo amplitude; and (v) estimation of the thickness of the corresponding ice layers, using eqn 2.

The average rate of growth was given by the observed growth of the ice layer (measured on both echographic lines) divided by the experimental time elapsed between the recording of the two events. Mean values and SD of the rate of growth of the ice layer are reported for each freeze site and each animal.

Operating the cryoprobe brought the surface of the ultrasonic transducer to about $-40^\circ C$ in less than 20 s (temperature measured with a thermocouple abutting the transducer active surface; Laplace, 1997). As damages of the

---

**Figure 3.** Schematic drawing of the method used to measure the ultrasonic velocity in frozen skin.

**Figure 4.** Principle of velocity measurements in frozen skin. (a) Echographic radiofrequency signal recorded before freeze initiation showing the cryoprobe echo signal $E_0$. (b) Echographic radiofrequency signal recorded during freezing, showing both the iceball wall echo signal $E_1$ and the cryoprobe echo signal $E_2$. Initiation of freeze. Each specimen underwent two freezing cycles, the second cycle being performed after complete thawing of the skin. Complete thawing was obtained in $\approx 30$ min.

Figures 3 and 4 illustrate the principle of velocity measurements in frozen skin. In a first step, the time of flight of the echo of the cryoprobe was measured on the echographic signal acquired before freezing. During freezing, the pulse first traveled through unfrozen tissue until the iceball boundary. Some of the energy was reflected ($t_1$ – time of flight of the pulse reflected at the surface of the freezing front) and some of the energy traveled forward until it reached the cryoprobe. A cryoprobe echo was generated. The time of flight $t_2$ of the echo of the cryoprobe during freezing was different from $t_0$ because of the velocity variations in the frozen tissue. The time of flight $t_0$ of the cryoprobe echo before freezing was compared with the time of flight $t_0$ due to the nonuniform temperature distribution in the tissue, the ultrasound velocity is a complex function of the spatial coordinates; however, for simplicity, temperature gradients were neglected and we considered, in the first approximation, that the partially frozen skin specimen consisted of only two different regions: a frozen region with constant velocity $c_f$ and an unfrozen region with constant velocity $c_u$. The velocity in the frozen tissue could then be derived using the following equation (Laugier and Berger, 1993):

$$c_f = c_u - \frac{t_0 - t_1}{t_2 - t_1} \quad (1)$$

Only the differences in times of flight were required. The time intervals $t_0 - t_1$ and $t_2 - t_1$ were determined by detection of the points of maximum amplitude on the corresponding waveforms. The value of velocity $c_u$ of normal pig skin was derived from previously reported measurements in the literature ($c_u = 1720 \text{ m per s}$; Goans et al, 1977; Cantrell et al, 1978). A value of ultrasound velocity of the frozen skin was derived from each echographic line recorded during the freezing cycle. Mean values and SD of $c_f$ are reported for each specimen and each cycle.

**In vivo monitoring of cryosurgery** Ultrasonic data were acquired during freezing cycles on three pigs (weighted $\approx 120 \text{ kg}$) under anesthesia. Experiments were carried out in the Laboratory of Applied Radiobiology (CEA, Gif-sur-Yvette, France). All the pigs were female; Large-White, and were provided by the collaborating laboratory (Authorization n°5564 of the Department of Agriculture, Forestry, and Fishing). Freezing was performed on several sites of the outer thigh of the pig skin (five different sites for pig 1, and six sites for pigs 2 and 3). These sites were preliminarily shaved. Pigs were anesthetized with an IM injection of Imalgene 1000 (ketamine: 4 ml) and placed under gaseous anesthesia (Halothan 2%, 66% oxygen, and 33% nitrous oxide).

The echographic cryoprobe has already been described above. It was used in combination with a high-frequency transducer. Because of the small thickness of tissue investigated (a few millimeters), a 20 MHz broadband transducer was selected. The characteristics of the transducer (Physical Acoustic, Princeton, NJ) were as follows: diameter of the active element, 3.6 mm; external diameter, 5 mm; center frequency, 20 MHz; –20 dB frequency bandwidth, 35 MHz; axial resolution, about 75 µm. Acquisition of ultrasonic data was performed using a DPR35 (JSR Ultrasonics, Pittford, NJ) pulser-receiver. The echographic signal was then digitized at a sampling rate of 100 MHz by an 8-bit dynamic range board SFT4100 (Sofrastet, Ecquevilly, France) and transferred to a PC computer for processing.

The echographic cryoprobe was coated with a contact gel. In response to the delivery of the cryogen in the cryoprobe, an iceball penetrated the tissue layer in contact with the cryoprobe surface and thickened gradually until the device was put in the thaw mode. To follow the kinetics of the iceball formation (i.e., size and rate of growth), a total of 200 echographic lines were recorded at a rate of one line per s during a freeze/thaw cycle. The freezing period lasted 110 s. Real-time estimate of the size of the growing iceball could be derived from each recorded echographic signal. The echo signal from the iceball wall was first identified (Fig 5), the time of flight of the echo signal $t_2$ was measured by detection of the point of maximum amplitude. Then, applying the equation:

$$\text{Thickness} = \frac{c_f t_2}{2} \quad (2)$$

where $c_f$ is the average value of ultrasound velocity of frozen skin previously determined, provided an estimate of the thickness of the ice layer.

The estimation of the average rate of growth of the iceball in the dermis (in mm per s) for each freeze site and each animal included several steps: (i) selection of one echographic line recorded shortly after initiation of freeze; (ii) selection of one echographic line recorded when the iceball wall had reached the dermis–hypodermis interface; (iii) identification of the echo from the iceball wall on the two echographic lines; (iv) measurement of the time of flight of these echo signals by detection of the maximum echo amplitude; and (v) estimation of the thickness of the corresponding ice layers, using eqn 2.

The average rate of growth was given by the observed growth of the ice layer (measured on both echographic lines) divided by the experimental time elapsed between the recording of the two events. Mean values and SD of the rate of growth of the ice layer are reported for each freeze site and each animal.

Operating the cryoprobe brought the surface of the ultrasonic transducer to about $-40^\circ C$ in less than 20 s (temperature measured with a thermocouple abutting the transducer active surface; Laplace, 1997). As damages of the
transducer could occur in response to fast cooling to low temperatures, the ultrasonic response of the transducer (center frequency, frequency bandwidth, and sensitivity) was regularly checked in order to ensure that it was functioning adequately and had not been damaged by previous test procedures at low temperatures. This was achieved by measuring the echo response from a stainless steel plate placed at a fixed distance from the transducer.

RESULTS

Ultrasound velocity of frozen pig skin

The results obtained for each specimen and for each freezing cycle are summarized in Table I. The results are listed for the five specimens in the first five rows. All data were based on averages of 100 consecutive measurements carried out in steps of 1 s on a given specimen for a given freezing cycle. The SD represents the “within-specimen variability” (i.e., the variability found between several recordings obtained from a single specimen and cycle). The sixth row contains pooled data of all specimens, the SD representing the “between-specimen” variability. We observed substantial variations of the velocity within samples, between samples, and also between freezing periods for each sample. Mean velocity values fell in the range 2763–3202 m per s for the first freezing period, and in the range 2698–3088 m per s for the second freezing period; however, the mean values of velocity, obtained by pooling the data from all the skin specimens included in this study, were similar for both freezing periods (2871 ± 174 m per s for the first freezing period, 2862 ± 166 m per s for the second freezing period).

In vivo monitoring of cryosurgery

The data collected during a freeze cycle at time intervals of 1 s are represented in Fig 6. The amplitude of the radiofrequency signals has been converted in gray level and plotted versus time (horizontal axis) in a TM (time–motion) echographic mode. Ultrasound propagation is represented on the vertical axis from the top to the bottom of the image. Experimental time is increasing on the horizontal axis from the left to the right of the TM image. Ultrasound data acquisition began a few seconds before initiating the freezing process. On the left side of Fig 5 are the radiofrequency signals recorded before freezing. The thickening ice layer is depicted during the freezing process by the echo of the freezing front (F), moving gradually from the upper left corner to the lower right corner (this freezing front moves away from the transducer). Note also that the echoes reflected at skin interfaces (E, epidermis–dermis interface; H, dermis–hypodermis interface) moved along the echographic signal during the freezing process from the bottom left side to the top right side of the image. Although the ultrasonic transducer remained at a fixed distance from the skin interfaces during the experimental procedure, the time of flight of signals reflected on the skin interfaces progressively decreased. This was a consequence of echographic signal distortion due to the increase of velocity inside the growing iceball. The magnitude of the freezing front echo was higher when it was located in the dermis than when it was located in the hypodermis. The cooling of the backing material of the ultrasonic probe to low temperatures inhibited its function of damping and resulted in an interfering echo signal reflected from the back wall of the backing (B).

The average rates of growth (mm per s) of the iceball in the dermis obtained for each animal are listed in Table II. All data are based on averages of several freeze sites. The SD represents the “within-animal variability” (i.e., the variability found between several freeze sites). The coefficient of variation (SD/mean) of the rate of growth for multiple tests carried out at different sites was ≈10%. The fourth row contains pooled data of all animals, the SD representing the “between-animal” variability. We observed substantial variations of the rate of growth of the iceball for different animals, the variability between animals being ≈20%. The average rate of growth of the iceball in normal pig dermis was found to be 0.1 ± 0.02 mm per s.

DISCUSSION

The values of ultrasound velocity of frozen pig skin specimens reported here were consistent with values reported previously by our group using a slightly different experimental set-up (Laugier and Berger, 1993).
Accurate knowledge of the specific value of ultrasound velocity in frozen tissues is a key feature for the success of the technique: the thickness of the frozen tissue layer is proportional to the velocity of frozen skin. Hence, a 10% accuracy error on the value of velocity will result in a 10% error on the estimation of the depth of the freezing front. The fact that the velocity was determined in partially frozen skin specimens could potentially bias the estimates. Average velocity values reported for each specimen were based on 100 averages derived from 100 consecutive echographic data recorded in steps of 1 s during the freeze period. It is well known that ultrasound velocity is a function of temperature. For example, in water, it increases by about 5 m per s per °C in the range of temperature near 0°C and by 3 m per s per degree in the range around 30°C (Del Grosso and Mader, 1972). Iceball growth and gradual tissue cooling occurring during the freezing period induced progressive changes of the temperature gradient within both the frozen and the unfrozen regions. Hence, this time-varying temperature distribution could have induced progressive changes in the values of \( c_u \) and \( c_f \), and could potentially be a source of uncertainty.

The speed of frozen tissue under conditions where the entire skin specimen was frozen would assure a more uniform distribution of sound speed and a more accurate estimation. A second technical problem was related to the precision of measuring time intervals \( t_0 - t_1 \) and \( t_2 - t_1 \) (eqn 1). The method of determining the time of flight relied upon determining the time of flight of the positive maximum amplitude of echo signals; however, signal interference such as those encountered under our experimental conditions and should be preferred for accurate estimates. The large variance of the results (≈5% of the velocity value) was partly due to these technical problems, and partly to inherent variability found between biologic specimens.

The echographic cryoprobe had a built-in high-frequency ultrasonic transducer that served two functions. It enabled \textit{in vivo} real-time monitoring of depth penetration of the iceball and gave important feedback to the operator or to the console relating to the rate of growth of the iceball. This feedback can be used to deliver a warning to the operator once the ice front has reached a predetermined depth. Automatic (i.e., operator-independent) detection of the echo signal from the freezing front and calculation of the depth penetration of the iceball was possible.

The magnitude of the echo signal was lower in the dermal layer than in the hypodermal layer (Fig 6). Hypodermis is constituted of multilayers of adipose tissue with a relatively small amount of water, which might explain the loss of intensity of the echo signal in hypodermis. The ability to detect the iceball wall under any clinical circumstances is an important characteristic of this technique, and attention should be focused on the optimization of the sensitivity of the ultrasonic transducer. Another technical problem was related to cooling of the backing material of the ultrasonic transducer; however, the length of the backing material can be adapted such that the interfering echo signal reflected from the back wall of the backing arrives later and thus cannot disturb the measurement.

The accuracy of the iceball thickness measurements is dependent upon three criteria: the accuracy with which the speed of sound of frozen skin is known; the accuracy of measuring the time of flight of the echo signal from the iceball boundary; and the absence of damage of the transducer due to fast cooling to low temperatures.

The speed of sound of frozen human skin must be known accurately before proceeding with clinical application. Our findings have enabled us to put forward an experimental protocol for the accurate estimation of the speed of frozen skin. It uses uniformly frozen skin specimens (e.g., using a cryostat) in a double transmission mode.

The accuracy of measuring the time of flight of the echo signal at the iceball boundary may be influenced by the distortion of the waveform induced by interfering echoes. This effect was not investigated in this study; however, it is reasonable to think that the uncertainty is within the range of the spatial axial resolution (≈75 μm) of the ultrasonic transducer.

Any damage caused to the transducer by low temperature may be a source of error for the measurement of the iceball thickness. Special care should be taken in the design of ultrasonic transducers for this particular application. The characteristics of the ultrasonic probe used in this study were frequently checked. The ultrasonic transducer appeared to be robust and had been operated for several hundreds of freeze-thaw cycles without any detectable damage, nor any variations of its frequency-dependent characteristics.

In order to check the absence of failure of the ultrasonic transducer and to ensure the reproducibility of the ultrasonic measurements, standard calibration procedures must be defined. These quality control tests should be regularly performed.

**CONCLUSION**

This study demonstrates the feasibility of \textit{in vivo} echographic monitoring of skin cryosurgery. Real-time processing of the radiofrequency signals, without sophisticated hardware implementation, provides the operator with the depth of the freezing front during the freezing process. Special care is needed in the realization of the ultrasonic transducers for this application. New data have been presented for the ultrasonic speed of normal frozen pig skin and for the kinetics of the growing iceball. Obviously our device has limitations, e.g., ultrasonic control of the growing iceball operates in a single direction along the transducer axis; however, small imaging transducers (motorized single-element probe or arrays of transducers) could potentially be combined with the cryoprobe tip that enables real-time cross-section echographic imaging of the iceball. In addition, with further research, one might envision using this kind of device with a cryoprobe driven by a different cooling agent (such as liquid nitrogen). Furthermore, attention should also be focused on the miniaturization of the echographic cryoprobe to potentially extend this technique to other clinical fields, including, for example, tracheobronchial tumor destruction or transcervical endometrial ablation.

**REFERENCES**


Graham GF: Advances in cryosurgery during the past decade. Castr 32:363–372, 1972


This work was supported partly by grant CRE 930905 from INSERM, partly by grant A93041649AT from ANVAR, and partly by grant 1363 from ARC.


