

A New Cryosurgical Device for Controlled Freezing

II. *In Vivo* Experiments on Skeletal Muscle of Rabbit Hindlimbs

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A new cryosurgical device was developed in this study to facilitate examination of factors affecting the outcome of cryotreatment. Special emphasis was placed on the control of the cooling rate at the freezing front. In the new computer-controlled cryosurgical device, the controlling variable is the cryoprobe temperature, which is calculated to ensure prespecified cooling rates at the freezing front. Details of the new cryodevice, results of a validation test, and the system characteristics are presented in Part I of this study. In this part of the study initial results of 13 *in vivo* experimental cryotreatments, including histological observations, are presented. The *in vivo* pilot investigations include the normal, healthy skin and the underlying skeletal muscle of the thighs in rabbits. Using low cooling rate-controlled freezing, the new cryosurgical device is demonstrated here as an effective surgical tool. An *in vivo* temperature measurement technique is employed based on miniature thermocouples and X-ray images. Thermal analysis of the heat transfer in the cryotreated tissue is presented, based on the temperature measurements and on numerical heat transfer simulations. Cryotreated tissue was extracted either immediately or 4 or 7 days following the procedure. The histological observations on the skeletal muscle of the 4- and 7-day postcryoinjury were not substantially different. The effective penetration depth of the cryolesion was in the range of 5-15 mm, possibly extending up to 25 mm, depending on the specific area treated and operating parameters. The cryotreatment resulted in complete destruction of cells in the skin followed by rapid replacement by epithelial cells. Histological responses to cryotreatment of skeletal muscle were similar to those resulting from a range of traumatic episodes, e.g., crush damage. It was also found that most of the blood vessels in the cryotreated region remained intact without histological evidence of extravasation of erythrocytes. © 1996

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Destruction of undesired biological tissues by freezing, termed *cryosurgery*, is considered to be an effective medical treatment modality (32, 34). Cryosurgery has several medical and economical advantages, e.g., low bleeding, good esthetic results, minimal use of anesthetics, short period of recovery, and low cost of the procedure (14, 15, 17, 18, 20). It has been reported that the cryotreatment often stimulates the immune system and thus activates the natural destruction mechanism of tumors and prevents their recurrence (1, 6, 37).

Criteria for the success of cryosurgery were suggested in several studies (22, 32). Among them are: the lowest temperature achieved (8),

the cooling rate during freezing (5, 10, 35), the thawing rate following the cooling process (16), and the number of repeated freezing/thawing cycles (10, 28).

A new cryosurgical device for *in vivo* experimental cryosurgery has been developed and is presented in the first part of this study (27). It is assumed in this study that the cooling rate at the freezing front and the repeated freezing/thawing cycles are among the most important factors affecting tissue destruction. In this new, computer-controlled cryodevice, the controlling variable is the cryoprobe temperature, and the temperature-forcing function is calculated to ensure specified cooling rates, slow or fast, at the freezing front. The cryodevice setup, results of validation tests, and system characteristics are presented in the first part of this study (27).

Operational capabilities of the new cryosur-

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gical device, which is an effective surgical tool using low rate-controlled freezing, are demonstrated in this part of the study. An *in vivo* pilot investigation is presented here, which includes the skin and the underlying skeletal muscle of rabbit hindlimbs. An *in vivo* temperature measurement technique is employed, using miniature thermocouples and X-ray images. A thermal analysis of the heat transfer in the cryotreated tissue is presented, based on the new temperature measurement technique and on numerical heat transfer simulations.

In vivo experiments related to this study were conducted by Rothenborg on rabbits and humans (29, 30) and on rats (31) and by Lenz and Eichler on rabbits (13). These investigators employed liquid nitrogen as the freezing agent in three different modes: direct spray, continuously cooled cryoprobe, and massive metal probes predipped in liquid nitrogen. They reported on the sizes of iceballs obtained, primarily as functions of cryoprobe diameter and of the method employed.

Rothenborg is mainly concerned with the mutual effects of the freezing on the microvasculature. The effects of a vasoconstrictor agent (epinephrine) on tissue and blood vessel survival are examined in some detail. Neither Rothenborg nor Lenz and Eichler were concerned with the control of the cooling rate although its importance has been recognized: "some sort of automatically controlled freezing system which correlates temperatures recorded from the tissue to a preset program of freezing and thawing" (30).

MATERIALS AND METHODS

The study was performed on four adult female and one adult male rabbits weighing 3–4.2 kg. The animals were maintained in conformance with the Guiding Principles in the Care and Use of Animals of the American Physiological Society. The experimental protocol received the approval of the Animal Welfare and Ethics Committee of the Technion Faculty of Medicine. The experiments were performed in the Animal and Experimental Surgery Unit of the Bruce Rappaport Faculty of Medicine. In total,

13 cryosurgical experiments were performed. Prior to experimentation, the fur was shaved from the areas to be treated. Anesthesia was induced by injections of ketamine hydrochloride (VMD, Veenendaal, The Netherlands), 30 mg/kg of body weight, prior to the cryosurgical treatment. The cryotreatment was performed on the lateral face of the thigh. The region affected by the cryotreatment included the skin, the hypodermis, underlying connective tissue, and skeletal muscle. The cooling rates in most of the experiments were 9°C/min, beginning at a normal tissue temperature and ending in the temperature range of –97°C to –126°C, depending on the specific experiment. The depth of penetration of freezing was in the range of 5–15 mm, depending on the cooling rate and the final cryoprobe temperature of a specific experiment. Additional preliminary cryosurgical experiments were performed on the skin above the lower back muscles. These latter experiments on the lower back proved unsuccessful because of the thick adipose layer of the hypodermis, which prevented penetration of the cryolesion to the underlying musculature. However, the skin of the back developed the same cryolesions as were found in the skin of the thigh treated by cryosurgery.

In the initial experiments sacrifice was performed about 15 min after completion of thawing at the end of the cryotreatment. In later experiments, sacrifice was performed at 4 and 7 days, respectively, after the cryotreatment. Sacrifice was performed by an overdose of ketamine hydrochloride. An area of 2–3-cm radius from the center of the probe was dissected out using sharp scalpels and scissors to a depth of about 1.5 cm. The skin tended to lose its relationship and separate from the underlying muscle. The area of the experimental freezing was fairly clearly demarcated because of the pinkish discoloration. Immediately after thawing, the area exposed to the probe showed edema, which became considerably more conspicuous 2–3 h after cryotreatment. The degree of edema was more severe when the region frozen was larger. The tissue was rapidly immersed and fixed in 10% neutral buffered form-

aldehyde for 2 days. The formaldehyde causes hardening of the tissue, which makes the subsequent trimming of the block easier. Using scalpels, the injured area was cut first in a longitudinal plane (to produce longitudinal histological preparations). One half of the tissue was further cut to produce blocks of muscle also in a transverse plane. The blocks were dehydrated in ascending ethanol concentrations, cleared in xylene, and embedded in paraffin wax using standard histological methods. Wax sections were cut on a microtome at $7\ \mu\text{m}$ and stained for histology using a routine hematoxylin & eosin technique.

In tissues taken shortly after the cryoinjury (15 min after thawing), the blocks of tissue proved virtually impossible to cut. The area of the injury did not produce sections but disintegrated and broke up into a powder-like material. This was apparently the result of the freezing injury, involving, among other factors, tissue desiccation and the biological destruction of the tissue that prevented proper infiltration of the wax. Blocks of tissue taken from the control

(contralateral) thigh, which was not treated with freezing, embedded well and showed no technical cutting problems. In contrast, blocks of tissue taken from rabbits 4 or 7 days after cryoinjury proved no problem in sectioning. In view of the technical difficulties involved in processing material taken shortly after cryoinjury, all of the histological observations were made on tissues taken 4 or 7 days following the cryotreatment.

HISTOLOGICAL OBSERVATIONS

Histological examination of the epidermis (the outermost layer of the skin, which was in direct contact with the cryoprobe) showed severe pathological damage at both 4 and 7 days posttreatment. The epidermis was irregular and thickened with marked indentations. The epidermis lost all of its normal cellular stratification (Fig. 1). The keratin layer was completely absent, and there were few signs of hair or hair components. The epidermis showed prominent infiltration of polymorphonuclear leukocytic cells in the injured region. It is possible that this

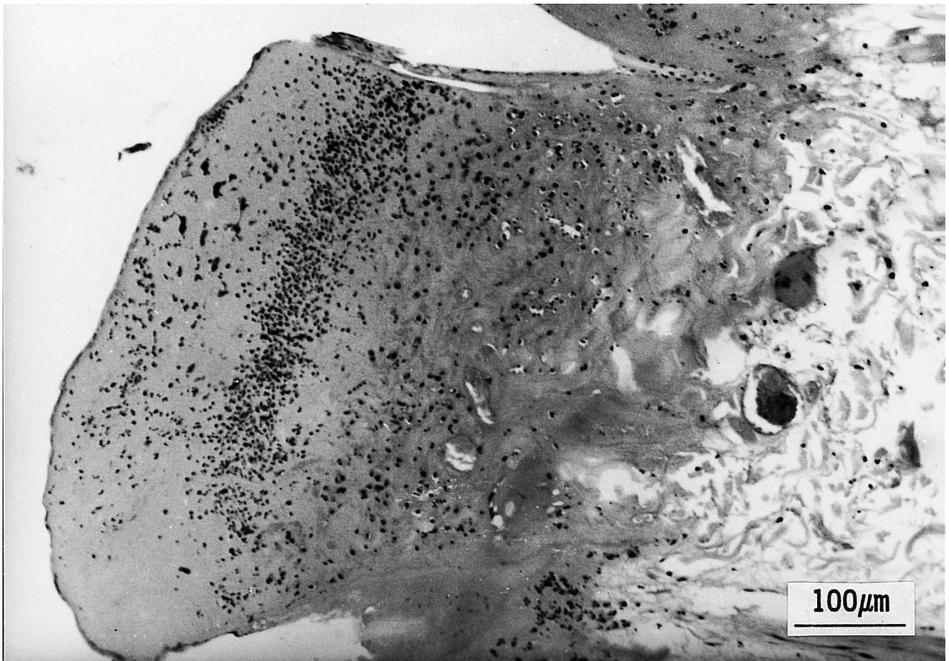


FIG. 1. Epidermis of the right thigh 4 days after cryotreatment. Severe pathological damage is found including the loss of normal stratification and the absence of keratin layer. The epidermis contains large numbers of infiltrating polymorphonuclear leukocytes. There are no signs of hair or associated structures.

epidermal layer, seen after 4 days, is replacement tissue derived from epithelium from areas adjacent to the cryoinjured region.

The skeletal muscle showed clear signs of histological damage at both 4 and 7 days post-cryotreatment. At low magnifications the injured area was easily detectable. Figs. 2–5 show the histological appearance of muscle (transverse sections) 7 days after cryotreatment. There was a gradual interface, of about 0.5-mm thickness, between the severely cryoinjured tissue and the surrounding normal (noninjured) muscle (Fig. 2). At the interface, muscle fibers appeared small, condensed, and shrunken with a clear region around them. In the main cryoinjured region this phenomenon was even more pronounced (Fig. 3 and Fig. 4). This clear region around each fiber appears to be due to accumulation of fluid (edema) resulting from the muscle injury (31). The connective tissue surrounding each fiber (endomysium) was very prominent (Fig. 4), and this was also the case with the perimysium (connective tissue surrounding bundles of fibers). Proliferating satellite cells and infiltrating macrophages (to clean

up the necrotic tissue) and polymorphonuclear leukocytes (associated with tissue damage or inflammation) were common in the connective tissue. The interface and cryodamaged regions also showed the development of newly formed myotubes with multiple nuclei (formed from aggregation of activated satellite cells). These myotubes are indicative of the early stages of muscle tissue repair. At higher magnifications cryodamage was clearly visible within fibers of the interface region (Fig. 5). This damage was nonhomogeneous within individual fibers. The histological observations on the skeletal muscle of both the 4- and 7-day postcryoinjury were not substantially different. Schematic description of the orientations of the histological preparations presented in Figs. 2–5 relative to the cryolesion are shown in Fig. 6.

In all of the histological preparations of the cryoinjured regions of the skeletal muscle, the blood vessels appeared intact and showed no indication of associated hemorrhage. However, many blood vessels showed signs of having become more irregular in shape. Similar observations were found concerning the blood vessels

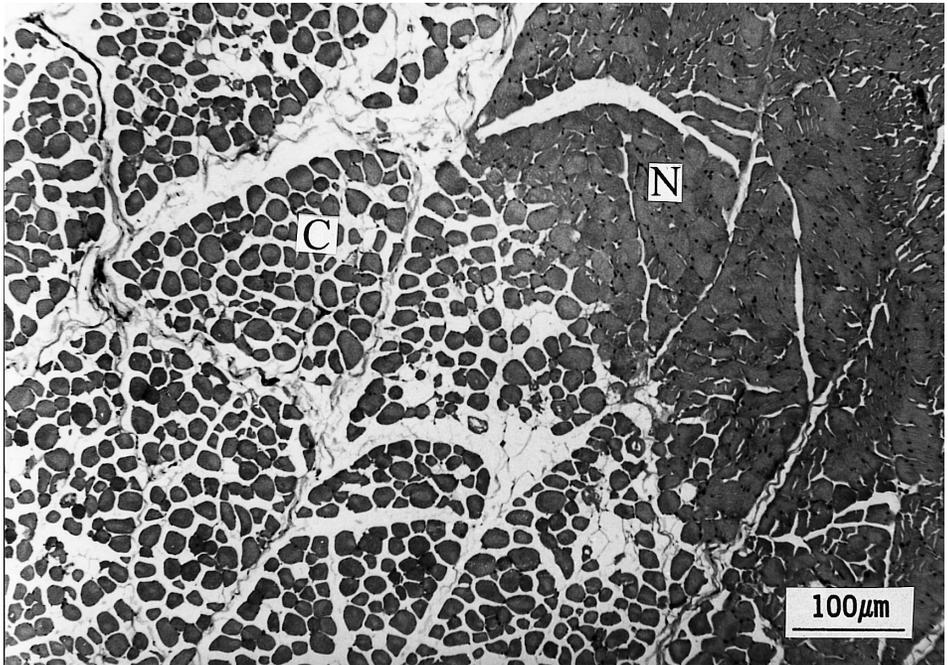


FIG. 2. Low power view of interface between normal (N) and cryodamaged fibers (C) 7 days after cryotreatment.

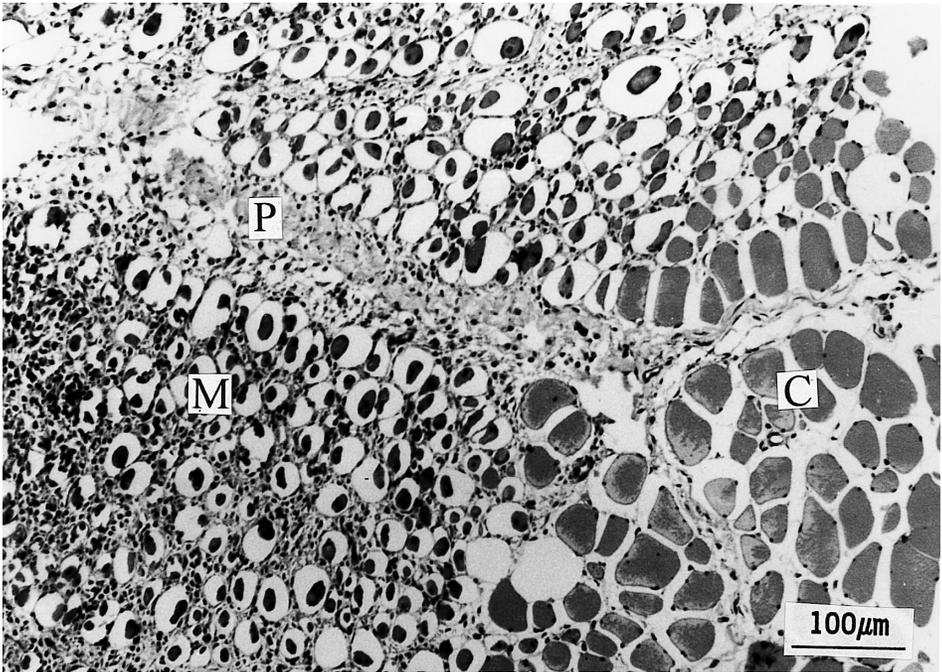


FIG. 3. Main cryoinjured region with severely damaged fibers (M) and the interface region with less damaged, more intact, fibers (C) 7 days after cryotreatment. Note the proliferation of perimysial connective tissue cells (P).

of the dermis and connective tissue under the cryoinjured skin. No endothelial cell loss of the blood vessels was observed in these experiments. Rothenborg observed complete and immediate resumption of blood flow following freezing in one study (29). In another study he indicated "severe damage to the vessel wall after cryosurgery-type freezing" based on an isotope assay (31).

IN VIVO TEMPERATURE MEASUREMENTS

In vivo temperature measurements in the biological tissue during cryotreatment may be useful for two different applications: 1) thermal analysis of the cryotreatment; and 2) as an extended feedback of the controlling system. Temperature measurements for thermal analysis purposes will be presented first.

Reliable *in vivo* temperature measurements are very difficult to perform for the following reasons. 1) Thermal conductivity of thermocouples is at least 1 order of magnitude higher than that of the biological tissue. Thus, thermocouples in the tissue may influence the heat

fluxes, on the one hand, and may increase the uncertainty of temperature measurements, on the other hand. 2) To decrease the uncertainty in temperature measurements resulting from the uncertainty in thermocouples placement, it is customary to use rigid supporting devices (2). These devices may, in turn, influence the heat fluxes, as mentioned above, and may also cause deformation to the soft biological tissue. 3) The penetration of thermocouples into the tissue may injure the living tissue, may cause bleeding, and thus may influence the extent of non-cryotreatment-related tissue destruction.

For thermal analysis purposes it is important to know the exact location of each thermocouple. However, it is less important to place the thermocouple in any specific preselected location. At first the thermocouple is placed in the target region of the tissue. As a second step, the exact location of each thermocouple is determined using X-ray images and a measuring tape. The location of the thermocouples, along with their temperature readings, are used as input data for the thermal analysis.

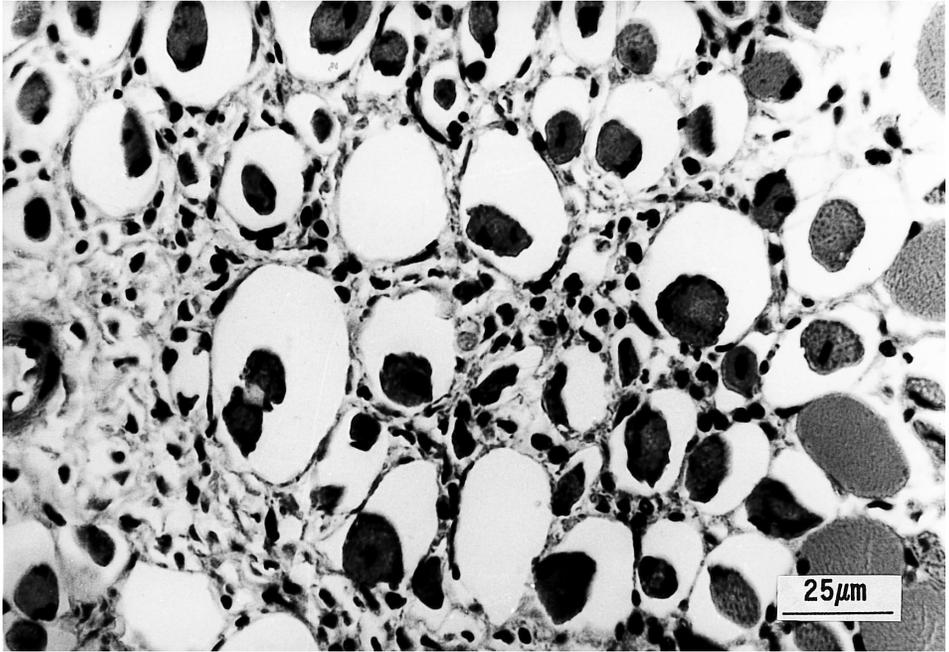


FIG. 4. Details of portion of the fibers of the main cryoinjured region seen in Fig. 3 7 days after cryotreatment. The fibers show marked shrinkage with a clear surrounding zone, typical of edematous tissue. Note the proliferation of the endomyosial connective tissue cells.

The miniature thermocouples used in this study are copper–constantan, each wire 0.1 mm in diameter, which are attached to a plastic fishing wire, 0.2 mm in diameter. A baseless needle, 1.2 mm in diameter and 10 cm long, is passed through the rabbit's hindlimb muscle, leaving the fishing wire inside the tissue with the thermocouple junction in the target region. Using this technique, the uncertainty associated with the placement of thermocouples is estimated as two to three times the thermocouple wires' diameter, i.e., 0.2–0.3 mm.

THERMAL ANALYSIS

The thermal analysis of the tissue–cryoprobe interaction is based on a total of 10 *in vivo* experiments on skeletal muscle of rabbit hindlimbs. Typical results from two experiments are presented, in which constant cooling rates of 9°C/min were effected. The two experiments started from initial temperatures of 36.8°C (Experiment A) and 34.5°C (Experiment B), at the measuring point, respectively. They were terminated when the cryoprobe temperature reached

−97 and −126°C, respectively. Three additional experiments, which are not included here, were performed on the skin covering the lower back muscles.

As presented by Rabin (23) and Rabin and Shitzer (24–26), all mathematical derivations are based on the bioheat equation:

$$C \frac{\partial T}{\partial t} = k \nabla^2 T + \dot{w}_b C_b (T_b - T) \quad [1]$$

where C and k are assumed to be constant and uniform in each phase. Additional nomenclature is shown in Table 1. Although this equation is not universally accepted as a complete model for heat transfer in biological tissues, we will show that it is nevertheless a good approximation for cases of freezing in peripheral tissues, characterized by a dense network of capillary blood vessels and low blood perfusion. It is noted that the metabolic heat generation effect is at most 1 order of magnitude smaller than the blood perfusion heating effect, and thus it has been omitted from Eq. [1].

Reliable thermophysical properties values are

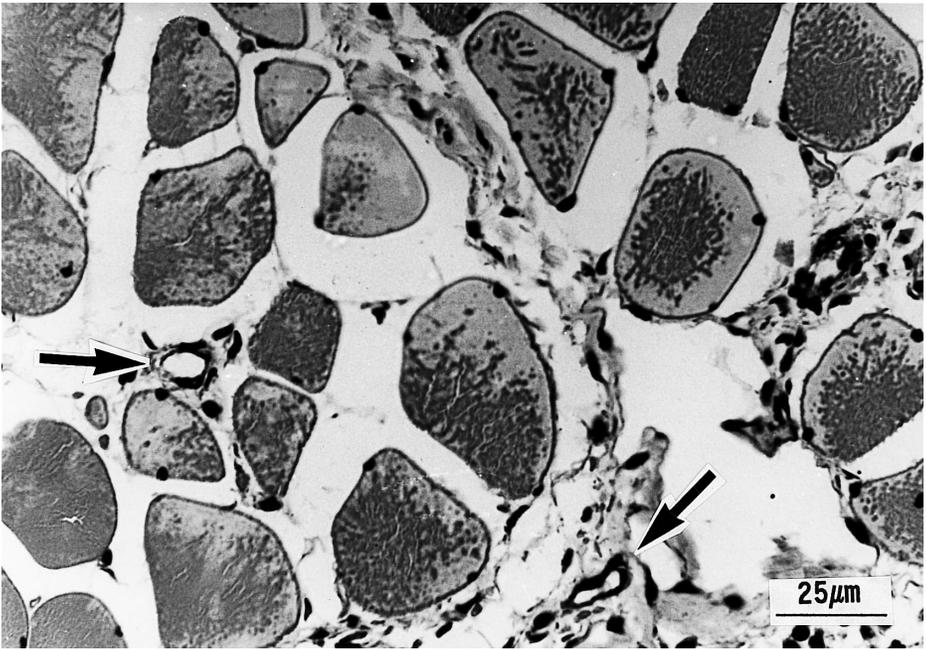


FIG. 5. Details of cryodamaged muscle fibers in the interface region 7 days after cryotreatment. The fibers are still intact, although their internal structure shows clear signs of cryodamage. The fibers have become separated from each other and surrounding clear spaces have developed (edema). Note the small blood vessels (arrows), which remain intact.

essential for obtaining accurate results from mathematical models. However, different tissues have different thermophysical properties, which are not generally available from the literature. In this study, the thermophysical properties were estimated using a least squares-based parameter estimation technique. The parameter estimation is based on experimental data and on two-dimensional and axi-symmetric numerical simulations. A target function is de-

finied for each point of experimental measurements in an individual experiment as follows:

$$\theta = \frac{1}{p} \sum_{p=1}^p (T_{TC} - T_{cal})^2 \quad [2]$$

where the time index p represents all available data at a certain point. The numerical simulations were repeated until the minimal value of

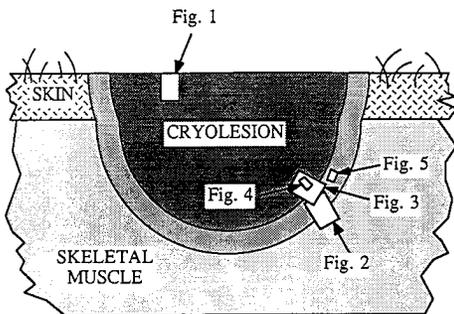


FIG. 6. Schematic description of the orientations of the biological preparations presented in Figs. 2–5 relative to the cryolesion.

TABLE 1
Nomenclature

C	Specific heat ($J/m^3 \cdot ^\circ C$)
k	Thermal conductivity ($W/m \cdot ^\circ C$)
t	Time (s)
T	Temperature ($^\circ C$)
\dot{w}_b	Blood perfusion (1/s)
θ	Target function for parameters estimation [$(^\circ C)^2$]
Subscripts	
b	Blood
cal	Calculated value
i	Index
p	Time Index
TC	Thermocouple

Eq. [2] was achieved. A minimum of the target function leads to the optimal parameters estimation with respect to a single measuring point in an individual experiment. Comparison of all parameters estimated, using data from all experiments, may obtain the best overall parameters estimation.

The heat transfer simulations were performed based on the following assumptions. 1) All thermophysical properties are uniform and constant in each region (phase). 2) Blood perfusion is uniform and constant in the unfrozen region. 3) There is no blood perfusion in the frozen region. 4) The thermal conductivity in the freezing region is an average value of the frozen and the unfrozen regions. 5) Phase transition occurs in the range of -1 to -8°C and reaches its peak at -3°C .

Thermophysical property values of water and ice were taken as the first guesses. The repeated parameters estimation process was carried out in the range of values presented in Table 2. It is interesting to note that parameters estimated from different measuring points in the medium and different experiments lead to approximately the same values. Average estimated values are presented in Table 2.

Fig. 7 and Fig. 8 present the comparisons between experimental data and computer simulations in Experiments A and B, respectively. The computer simulations are based on the average thermophysical properties presented in Table 2. The average value of the absolute errors, at the measuring points presented in these

figures, are 0.7 and 1.05°C , respectively. The target function values at these points, calculated by Eq. [2], are $0.73(^{\circ}\text{C})^2$ and $1.22(^{\circ}\text{C})^2$, respectively. Fig. 6 illustrates the uncertainty in temperature measurements resulting from the uncertainty in the thermocouple placement.

The process of parameter estimations is limited for the following reasons. 1) The estimated thermophysical property values are actually average ones in the entire thigh. 2) Tissue contraction, both as a result of the physical phenomenon of phase changing and as a physiological response to low temperatures, was neglected. 3) the parameter estimation is based on Pennes' bioheat equation, which might not be the perfect mathematical model. However, the mathematical model and the parameter estimation proved to be good approximations for engineering calculations. The temperature field, and thus the freezing front location, may be estimated reliably using the technique presented here. Moreover, the estimated parameters may be used for the cryoprobe-forcing function solutions in subsequent experiments in the same organ.

DISCUSSION

Cryosurgery has proven to be a relatively simple, painless, bloodless, and safe method for tissue destruction and is increasingly used in clinical environments to treat a variety of disorders, in particular with regard to the reduction or total ablation of benign or malignant tumors (9–11, 19, 28). *In situ* freezing techniques are

TABLE 2

Typical Thermophysical Properties of Biological Tissues (23) and the Results from the Parametric Estimation Process

Thermophysical property	Biological tissue	Parameter estimation
Upper temperature bound of Freezing	-1°C	-1°C
Peak of phase transition temperature	-3°C	-3°C
Lower temperature bound of freezing	-8°C	-8°C
Thermal conductivity in unfrozen region	0.385–0.63 W/m $^{\circ}\text{C}$	0.63 W/m $^{\circ}\text{C}$
Thermal conductivity in frozen region	1.3–2.25 W/m $^{\circ}\text{C}$	1.51 W/m $^{\circ}\text{C}$
Volumetric specific heat in unfrozen region	3.0–5.14 MJ/m $^3\cdot^{\circ}\text{C}$	3.16 MJ/m $^3\cdot^{\circ}\text{C}$
Volumetric specific heat in frozen region	1.13–2.0 MJ/m $^3\cdot^{\circ}\text{C}$	1.93 MJ/m $^3\cdot^{\circ}\text{C}$
Latent heat of solidification	233–300 MJ/m 3	300 MJ/m 3
Product of blood perfusion and its specific heat	0–25 kW/m $^3\cdot^{\circ}\text{C}$	20 kW/m $^3\cdot^{\circ}\text{C}$

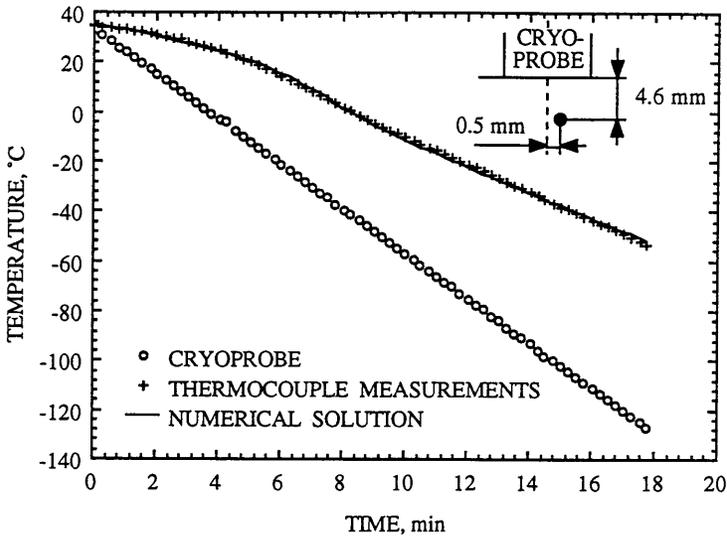


FIG. 7. Comparison of numerical results based on estimated parameters and measured data from Experiment A. The estimated thermocouple location is shown in the inset.

highly successful in causing local necrosis especially in tissues that are superficial and easily accessible including the skin, oral cavity, and uterine cervix (11). Cryosurgery has been used in treatment of internal organs such as the liver, prostate gland, lung, and breast (33). The aim in all of these cryosurgical procedures is the destruction of abnormal or pathological tissue in a

limited region, with minimal damage to the surrounding normal healthy tissues. One of the advantages of cryosurgical intervention over more traditional surgical procedures is the resulting minimal scar tissue, which is an important consideration for esthetic or cosmetic reasons in treatment of superficial tissues such as the skin. In view of the fact that freezing has an anes-

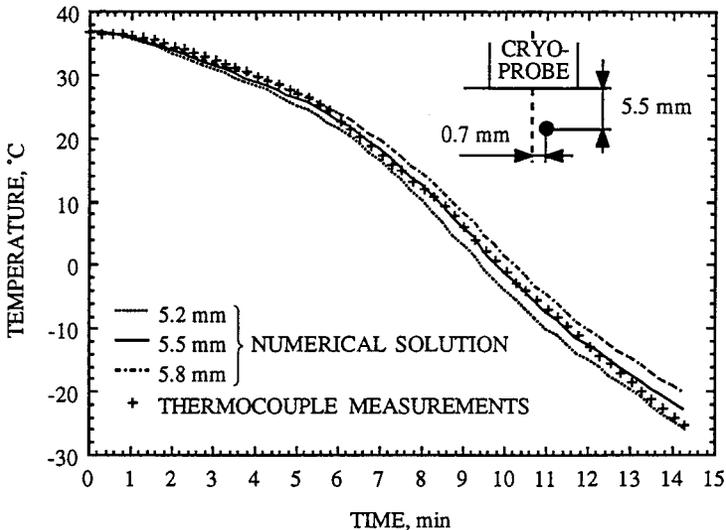


FIG. 8. Comparison of numerical results based on estimated parameters and measured data from Experiment B. The estimated thermocouple location is shown in the inset.

thetic effect, the anesthesia in many cases can be local, with the advantages this permits. The use of cryosurgery in practical terms often results in minimal hospitalization and early post-operative release.

Cryosurgical procedures in practice today do not commonly employ specific control of the time-dependent temperature of the cryoprobe. This, when applied, enables accurate control of the freezing rate, the warming and thawing processes, and of successive freezing/thawing cycles under similar conditions. Analogous to single cell destruction in cell cultures, we believe that the cooling rate at the freezing front is one of the most important factors of the destruction process, although this is not conclusively proved with regard to *in vivo* cryosurgery of animal tissues. Furthermore, and based on heat transfer considerations, accurate control of the temperature of the cryoprobe can increase the temperature gradients within the region undergoing phase transition. This, in turn, will decrease the thickness of the freezing region and thus increase the cryodestruction at the edge of the cryolesion, hence the motivation for the development of the new controlled cryosurgical device as described in a previous paper (25).

Our preliminary results in rabbits confirm that our experimental apparatus is an effective cryosurgical instrument for inducing limited cryolesions in both superficial tissues (the epidermis of the skin) and in deeper lying skeletal muscle at low freezing rates at the freezing front (less than $12^{\circ}\text{C}/\text{min}$). We have shown that the effective penetration depth of freezing under the controlled cryoprobe extends up to 15 mm. However, we estimate the maximal depth as 25 mm. Moreover, we have also shown that the boundary (interface) between the frozen and unfrozen regions is relatively narrow (about 0.5 mm). This interface has been shown to be easily detectable following the cryotreatment, both macroscopically, as seen immediately after thawing, and microscopically in histological sections in the postcryoinjury recovery period.

The histological changes seen in the epidermis show that the original tissue in the cryotreated area has undergone complete de-

struction. Rapid replacement by epithelial cells ensued, presumably from the areas adjacent to the cryoinjury. These epithelial cells need to cover the injured area as soon as possible, to provide a functionally defensive role in particular in preventing the loss of tissue fluids. Four and 7 days following the cryoinjury the epithelial cells have still not reorganized to their normal stratified condition, (Fig. 1). Moreover, the hairs and associated glands of the dermis are absent. The presence of large numbers of infiltrating cells, in particular the polymorphonuclear leukocytes, is common in any traumatized tissue and is one of the body's defense mechanisms in response to damage and potential infection. These are usually accompanied by macrophages involved in phagocytosis of necrotized cells or cell components. As the epidermis is composed of epithelial cells with very rapid rates of proliferation owing to their key defensive function, it is highly likely that if the period of recovery after cryotreatment were prolonged, the epidermis would regain its normal histological appearance, including the reestablishment of associated epithelial elements, e.g., hairs and associated glands of the dermis.

The histological responses to cryotreatment of the skeletal muscle resulted in effects similar to those resulting from a range of traumatic injuries including: crush damage, chemical poisoning, surgical excision, and excessive exercise (3, 4, 7, 12, 21). It would appear that a common morphological response occurs in skeletal muscle regardless of the type of injury or insult. Even 4 days after the cryodamage, there are already early indications of regeneration, including the formation of new myotubes. There are relatively few reports of experimental freezing damage to skeletal muscle in the literature, and in all these cases the freezing was fast and uncontrolled (21, 36).

One of the advantages of cryosurgery over other surgical procedures is the virtual absence of hemorrhage in the region of the lesion. The cryotreatment in our experiments was performed in selected regions in which major blood vessels were absent. The presence of large blood vessels would probably have affected our

observations and interpretations of controlled freezing. It is known that major blood vessels with blood flowing in them at the time of freezing are protected during cryosurgery (19). This can be highly advantageous in permitting the cryodestruction of tumors abutting major vessels without sacrificing the vessels. In our experiments only very minor freezing damage was seen in the small blood vessels: arterioles, venules, and capillaries of the dermis and underlying skeletal muscle. In transverse sections the endothelial cells lining these small blood vessels developed irregular profiles. However, most of the blood vessels appeared to remain intact after the cryotreatment. There were no histological signs of extravasation of erythrocytes, although the edema seen initially following the cryoinjury was apparently the result of temporary damage or leakage from the smaller blood vessels and in particular the capillaries, which is common in most cases of trauma. We have strong evidence suggesting that most or all of the blood vessels in the frozen region were definitely frozen, although upon thawing they appeared to remain intact and have their functionality restored. It appears that most of the vessels survive the cryotreatment, though we do not know if some of the vessels were destroyed as a result of the freezing and if any revascularization has occurred. The survival of most of the blood vessels appears to be an important factor in the repair and reconstruction processes following slow controlled cryotreatment and in particular with regard to the skeletal muscle.

One of the major limitations in cryosurgery associated with controlled freezing involves the difficulties in accurate monitoring of the extent of the freezing *in situ*. Ultrasound is a widely accepted technique for locating and following the freezing front (19). However this monitoring technique fails in some cases such as breast cancer. Recently, various imaging techniques—computerized tomography (CT) and magnetic resonance imaging (MRI)—have been introduced as possible means of monitoring the freezing process and the extent of the cryoinjury (33). However, it is still not really practicable to adopt these sophisticated and expensive imag-

ing systems for monitoring cryosurgery in clinical practice. In the present study, a new different approach was applied for the estimation of the freezing front location. This was estimated using real-time heat transfer simulations of the cryoprocure and an approximate model of the heat transfer in the biological tissue. The reliable thermophysical properties values needed for this application were estimated using experimental data and a least squares method for parameters estimation, as discussed above.

An alternative approach for monitoring the freezing process is associated with *in vivo* temperature measurements. Miniature temperature sensors are placed at critical points around the frozen region and act as extended feedbacks to the control system. Temperature readings from these sensors may indicate the freezing of the entire target region and thus the completion of the cryoprocure. Alternatively, the miniature sensors may be placed at the borders of vital tissues surrounding the target region. The exact location of the thermocouples is determined by X-ray images, as discussed above.

CONCLUSIONS

We have demonstrated the effectiveness of the new cryosurgical device, involving controlled low rate of freezing, in cryotreatments which include the skin and the underlying skeletal muscle of hindlimbs of rabbits. Very little is known concerning controlled freezing injuries to muscle tissues. These pilot studies show that cryodamage is so severe in the immediate postinjury period, that normal histology cannot be successfully performed, although after 4 or 7 days there are already signs of early recovery and regeneration.

The morphological indications of skeletal muscle injury and early regeneration seem similar regardless of the method of injury. Muscle tissue injury is characterized by necrosis, revascularization, infiltration of inflammatory cells, phagocytosis of the necrotic muscle tissue, proliferation of mononuclear muscle precursors, and their fusion to form multinucleated young muscle cells called myotubes.

An *in vivo* temperature measurement tech-

nique has been employed in this study, using miniature thermocouples and X-ray images. Temperature sensors placed by this new technique have been used for thermal analysis of the cryotreatment.

Thermal analysis based on the bioheat equation and parameter estimation has been performed. The bioheat equation may not be the perfect mathematical model; however, this equation and the parameter estimation proved to be good approximations for engineering calculations. This provides a rather reliable means of calculating the temperature field in the tissue and for estimating the location of the freezing front.

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