The Effect of Immediate Sympathectomy on Tissue Survival Following Experimental Frostbite *

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THE TREATMENT of freezing injuries has been in dispute for many years. Therapy has often been based more on folklore than on experimentation. Apparently logical thinking and seemingly reasonable analysis often led to conclusions which have since been proved erroneous by controlled experimentation. It was only after the great interest in cold injuries aroused by the Second World War and the Korean conflict that strides were made toward well documented evaluation of therapy.

One great advance has been the demonstration, both experimentally [9, 11, 15] and clinically,[26, 28, 31] that rapid thawing of frozen tissue improves the degree of tissue survival considerably. This demonstration makes untenable the previously held position that rapid rewarming should not be used in frostbite management because it produces greater pain and edema [30] than slow warming.

However, the treatment of freezing injuries is still not satisfactory, no combination of current therapeutic measures being sufficient to allow complete anatomical and functional repair.[26, 31] One of the various types of adjunct therapy [26, 27, 28, 31] which have been recommended is interruption of the nerve supply to the vasculature of the injured and adjacent areas [2, 5, 7, 9, 10, 20, 21, 22, 29, 30] by sympathetic block or surgical sympathectomy.

The following paper describes a study of the influence of *immediate* surgical sympathectomy on tissue survival following freezing injury in experimental animals. No attempt has been made to evaluate any other form of treatment. The value of sympathectomy in the treatment of the late sequellae of frostbite has generally been accepted [7, 22] and is not evaluated here. All feet were thawed rapidly because, in the authors' opinion, this procedure has been demonstrated to be of benefit and, if possible, should not be omitted from any therapeutic regimen now available, regardless of other forms of treatment employed.

Material and Methods

Seventy-four NMRI New Zealand rabbits weighing between 2,500 Gm. and 3,100 Gm. were exposed to the following procedure: They were anesthetized with intravenous pentobarbital, 35 mg./Kg. initially and additional doses as needed. The latter was administered through an intravenous saline drip into an ear vein. Each animal received a total of 30 to 100 cc. of saline during each procedure. Both hind feet were shaved with electric animal clippers to a level above the ankle and then depilated with a commercial depilatory, Nair®. A copper-constantan thermocouple was in-

* Submitted for publication July 1, 1963.

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The opinions or assertions contained herein are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.

serted into the tissue of the left foot between the second and third metatarsals just above the metatarsophalangeal joint, so that the thermosensitive junction lay equidistant from the volar and plantar surfaces at that point (Fig. 1). Rectal temperature and the temperature of the immersion baths were also measured with copper-constantan thermocouples. All temperatures were recorded on a Minneapolis-Honeywell Electronik Brown 12-Channel Recorder.

The left foot was immersed to the level of the process at the proximal end of the lateral metatarsal (Fig. 1) in an ethylene-glycol-alcohol-water mixture maintained at −15° C. by the addition of solid carbon dioxide; the bubbling thus produced also

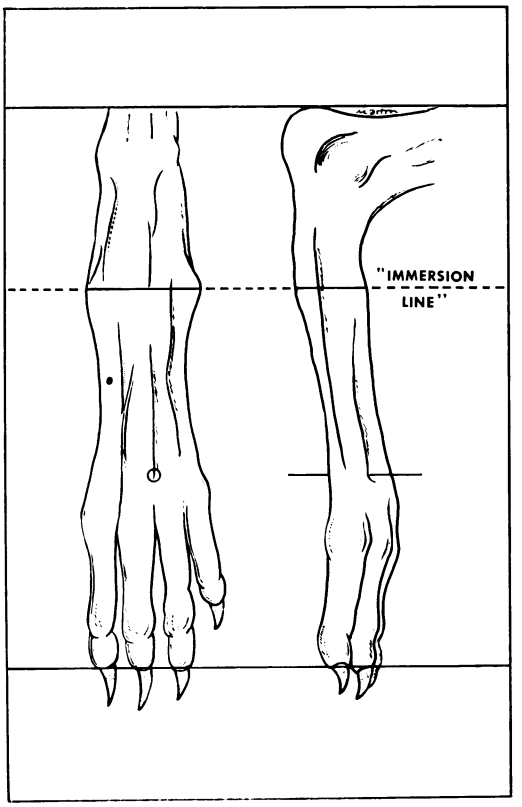

FIG. 1. Depilated left hind foot of rabbit. Note the level to which the foot was immersed. The thermocouple was inserted between the base of the second and third toes.

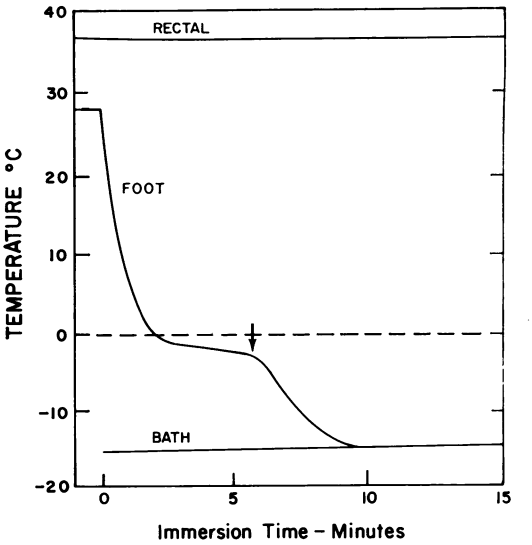

FIG. 2. Temperature of the foot during freezing. The foot was immersed at time *O;* the arrow indicates when freezing was considered to be complete.

kept the bath stirred. The time when freezing was complete was determined by the temperature curve, as recorded by the thermocouple within the foot (Fig. 2). Upon immersion, the temperature rapidly fell to the freezing point of tissue, about −0.5° C. There the curve leveled out and changed little for several minutes. This represented the period of time during which latent heat of fusion resulting from ice formation was being lost to the cold bath.[25] At the point indicated by the arrow (Fig. 2), freezing was considered to be complete, and the temperature once again fell at about the same rate as before.

Some feet supercooled before they froze, as shown in Figure 3. In this case, the temperature did not level out initially at the freezing point, but dropped in a smooth curve to as low as −10° to −14° C., and then, suddenly, jumped back up to the freezing point of tissue. At this moment, the tissue became hard and white and appeared clinically frozen, and the animal, although well anesthetized, showed a flexor response as though reacting to a painful

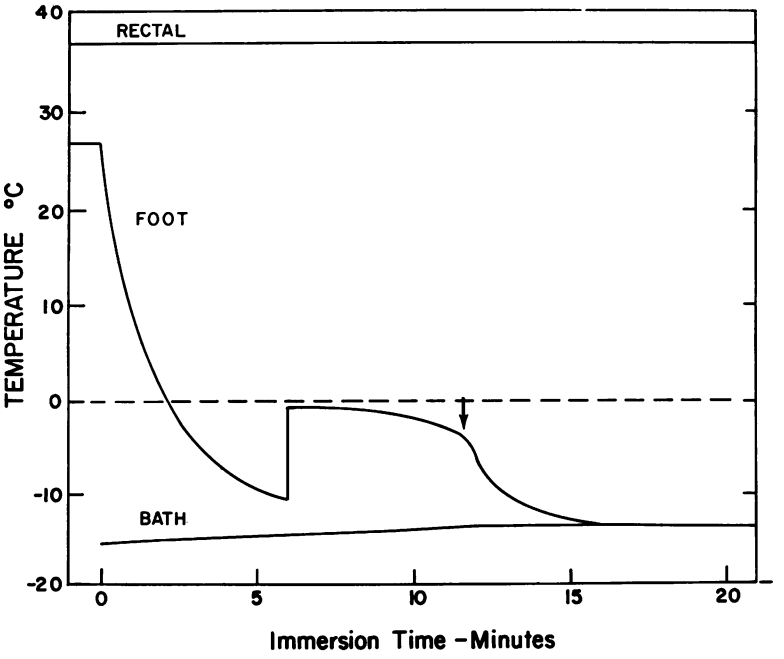

FIG. 3. Typical temperature curve of foot during supercooling. Note the sudden rise in temperature to the freezing point at about six minutes. The arrow indicates when freezing was considered to be complete.

stimulus (cf. Lewis's *pricking sensation*[23]). Theoretically, at that moment, there was sudden formation of small ice crystals throughout the supercooled tissue, accompanied by a release of latent heat of fusion which reheated the mass back to near 0° C.[6] As the ice crystals then grew slowly, the freezing pattern continued as described above, and the process was considered complete at the point indicated by the arrow in Figure 3.

The foot was kept in the cold bath for 18 minutes from the point indicated by the arrow and was then immediately transferred to a water bath at 42–43° C. It was kept in the warm bath until one minute after foot temperature had attained the same level as rectal temperature. The foot was then gently dried and left exposed to room air (25.0–27.5° C.).

Sympathectomy was performed either before or after freezing, using pentobarbital anesthesia. In all cases the left lumbar sympathetic chain was excised, from about the second to the fifth lumbar segment. Successful sympathectomy was confirmed by histological section of the excised tissue, in order to identify the appropriate number of ganglia.

All rabbits were given Bicillin, 2 cc. (1,200,000 units), into the right hamstring muscle group at the conclusion of freezing or sympathectomy, whichever was performed first. In addition, approximately two teaspoons of veterinary oxytetracycline were added to each gallon of drinking water for a week before and during the experimental period.

The rabbits were observed periodically for 30 days following freezing, by which time healing of the stump was well under way and there was no further tissue loss. At that time, linear measurements of surviving tissue were made from the immersion line to the end of the stump and compared to the total length of the immersed segment. The ratios were expressed as per cent linear tissue survival.

The rabbits were divided into four groups, depending on when sympathectomy was performed in relation to the time of freezing and thawing. Forty rabbits were

included in this study. Group I, a control of 11 rabbits, had no sympathectomy. Group II, ten rabbits, had sympathectomy three days prior to the freezing injury. Group III, nine rabbits, had sympathectomies within one hour after freezing and thawing. The animals in Group IV, ten rabbits, were sympathectomized approximately 24 hours after freezing and thawing.

Results

Results are summarized in Table 1 and Figure 4.

Mortality. Seventy-four rabbits received freezing injuries, but 34 were not included in the statistical evaluation. The reasons for exclusion varied: one rabbit chewed through the incision sutures; one died from acute urinary retention; one from respiratory disease; ten died during the sympathectomy operation; eight from unexplained cause; and 11 died or were sacrificed because of a broken extremity or symptoms of a broken back. The actual mechanism of these last injuries is not definite, but one possibility is that claws of the anesthetic frostbitten foot caught in the screening of the floor of the cage and the rabbits injured themselves while trying to pull free.

Two rabbits were excluded from the series because of an unusual physiologic response to immersion in the freezing bath. These rabbits supercooled and rewarmed their feet so efficiently that one finally froze only after 45 minutes in the cold bath and the other after 76 minutes (vs. 6.6 minutes over-all average for the other rabbits).

Tissue Survival. There was no significant difference in linear tissue survival between Groups I, II, and IV, i.e., between the controls, the animals sympathectomized three days before freezing, and those sympathec-

TABLE 1. *Tissue Survival and Freezing Characteristics of Rabbits' Feet*

	No. of Animals	Supercooling Incidence %	Linear Tissue Survival %*	Time to Freeze (seconds)*
Group I (control)				
All animals	11	36	54 ± 14	
Supercool	4		51 ± 12 } p = 0.30	
No supercool	7		56 ± 16 }	480 ± 115
Group II (Sympathectomy three days prior to frostbite)				
All animals	10	70	55 ± 23	
Supercool	7		56 ± 28 } p > 0.90	
No supercool	3		55 ± 4 }	307 ± 40
Group III (Sympathectomy immediately following frostbite)				
All animals	9	56	71 ± 21	
Supercool	5		61 ± 18 } p < 0.01	
No supercool	4		83 ± 19 }	383 ± 83
Group IV (Sympathectomy 24 hours following frostbite)				
All animals	10	40	58 ± 24	
Supercool	4		45 ± 26 } p < 0.01	
No supercool	6		67 ± 20 }	424 ± 124
Group I + II + III + IV				
All animals	40	50		
Supercool	20		54 ± 21 } p < 0.01	
No supercool	20		65 ± 19 }	

* All figures indicated by ± represent standard deviations.

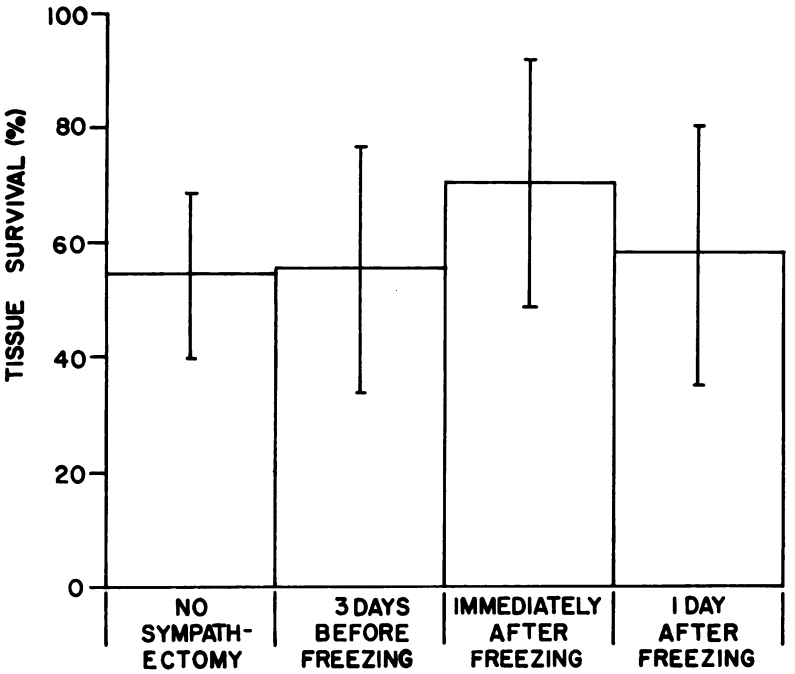

FIG. 4. Tissue survival expressed as per cent of length of immersed segment which survived after 30 days. Vertical lines represent one standard deviation.

tomized one day after freezing. In Group III, however (rabbits sympathectomized immediately after freezing and thawing), the linear tissue survival was greater than that in the other three groups, with a statistical significance at about the 6 per cent level by the chi-square test (Table 1 and Fig. 4). Note that there was an increase in tissue survival following freezing injury only if sympathetic denervation immediately followed freezing and thawing. Sympathectomy performed three days before or 24 hours after freezing injury did not enhance tissue survival.

Supercooling. Half of the 40 animals included in the final series demonstrated the phenomenon of supercooling, as shown in Table 1. Note, however, that 70 per cent of the animals sympathectomized before freezing (Group II) demonstrated supercooling as opposed to only 43.3 per cent of the rabbits in the other three groups combined. Because of the small numbers involved, this apparent trend is not statistically significant, and, indeed, analysis of the entire group of 74 rabbits verifies that this is probably only a result of sampling variability.

One may ask whether the occurrence of supercooling had any effect on subsequent tissue survival. It apparently did, as there is significantly more tissue loss associated with supercooling if all the animals are considered (Table 1). When taken as groups, only III and IV showed more damage in supercooled feet, but the numbers in the subgroups are hardly large enough to be statistically valid.

Time Required to Freeze. The time from immersion to completion of freezing was recorded in all experiments. Because of the great difference in heat transfer characteristics between non-frozen tissue and tissue impregnated with ice cystals,[25] however, one cannot directly compare the time required for freezing in the rabbits that froze normally and those that supercooled. If only those animals not demonstrating supercooling are considered, the average time required to freeze was shorter for Group

II (the animals which were sympathectomized before freezing) than for those animals which had not undergone sympathectomy prior to freezing. Despite the small numbers in the samples, the shortened time required to freeze a foot deprived of sympathetic innervation is statistically significant; this significance is increased when we include the animals which were well at the time of freezing, but which died later of causes indirectly related to the freezing injury.

There is no statistical correlation between the length of time required for the foot to freeze and later tissue survival. Since the length of time required to freeze determines the total immersion time, it follows that there is no correlation between total immersion time and tissue survival. This once again demonstrates the dangers in attempting to produce a standard freezing injury by immersing an extremity in a cold bath for a standard period of time, instead of measuring tissue temperatures to determine when freezing actually takes place and timing immersion from that point.[14, 15, 20]

Discussion

There have been numerous favorable reports on the use of surgical sympathectomy in the treatment of the *late sequellae* of frostbite.[22, 29, 30] These authors noted great improvement in the hyperhidrosis, cold and pressure sensitivity, and vascular lability which often follow a freezing injury by several months and become a persistent chronic condition.

The use of sympathetic denervation in the *immediate* therapy of freezing injuries has not, however, been widely used. The procedure was rejected by most clinicians without trial for many years, partly because of the quasilogical conclusion that freezing itself renders the innervation in the frozen area nonfunctional and further interruption of the autonomic nerve fibers would add nothing.[22, 30] However, the few authors who have had experience with surgical sympathectomy, or sympathetic block performed within a few days following the acute injury, are quite enthusiastic in recommending it.[2, 7, 21, 30]

The evaluation in animals of the effect of sympathectomy on standardized experimental frostbite has been inconsistent, some reports claiming great increase in tissue survival with the use of sympathectomy and others reporting no effect or even increased loss. On closer inspection, however, the controversy is not so great as it seems at first glance. In freezing injuries of the lower extremities of the rabbit or dog treated with lumbar sympathectomy, the experiments generally revealed an increase in tissue survival in animals with relatively early sympathetic interruption.[9, 20] However, freezing injury in rabbit ears made in conjunction with cervical or stellate sympathectomy demonstrated as much as or more tissue loss than that found in control ears.[9, 10, 16] This discrepancy in the efficacy of sympathectomy between freezing injury in ears and in lower extremities may depend on the fact that the vasculature of the rabbit ear probably functions as a specialized structure of temperature control with an active, but not necessarily representative, sympathetic innervation.[12]

The use of sympatholytic drugs, sympathetic blocking agents, and vasodilators has been evaluated experimentally,[9, 10] and, in general, some increase in tissue survival has been seen, adding evidence that interruption of sympathetic control favors tissue survival.

Many aspects of the pathophysiology of freezing injuries have been presented by numerous authors.[1, 2, 11, 13, 14, 15, 17, 18, 21, 22, 23, 24, 25, 26, 29, 31] Although there is controversy concerning many details of the reaction of tissue to freezing and thawing, the following picture is generally presented:

Upon freezing, ice crystals form in the tissues. The size and location of these crystals are de-

pendent in general on the speed of freezing.[25] Most clinical frostbite occurs at slow rates of freezing, during which extracellular crystal nuclei form and grow, leaving the intracellular spaces relatively free of ice crystals. As the extracellular crystals grow, they incorporate more of the available free water, leaving the unfrozen portion of the extracellular medium hypertonic. Meanwhile, free water is drawn out of the cells and added to the growing extracellular crystals leaving the intracellular space, as well, hypertonic, probably to a toxic degree. Although great cellular distortion can be seen during freezing, it is highly unlikely that cell membranes rupture during this process.[25]

During thawing, the water is once again distributed between the extra and intracellular spaces. Probably some tissue damage occurs during the freezing phase and frozen state,[11, 26] but most injury occurs during and shortly after thawing.[2, 16, 21, 26, 29, 31] The effect of the thawing process depends both on the rapidity with which the blood flow returns and the rate of increase in tissue metabolism. It is of dubious benefit for one to raise the rate of metabolism slowly and carefully (as by slow thawing technics) if in doing so one retards the return of blood supply to such an extent that it is inadequate for the increased metabolism. Apparently 42–43° C. is the temperature range at which the rate of thaw is most favorable to balance the increase in metabolism with return of blood supply, and yet not exceed the temperature at which direct heat damage occurs.[11]

Immediately after thawing, there is a transient vascular spasm[2] followed usually within a few minutes by resumption of blood flow.[1, 2, 14, 16, 22, 24] However, within a few more minutes, although the gross appearance of the extremity is one of hyperemia,[19] the flow in the capillaries in the frost bitten tissue slows and finally ceases.[1, 4, 16, 18, 19] During this time there is a massive exudation of fluids through the capillary wall, and the stagnant red blood cells are left behind in a *sludged*[16, 17] or *silted*[13] state. At this point, these small vessels can be washed out by infusion of saline or return of blood flow,[16] but if the situation remains uncorrected, thrombosis ensues.[18] While the flow slows, the arterioles and venules in and around the frostbitten tissue proceed into spasm[2, 16, 18, 29] and arteriovenous shunts open.[1, 18] By the end of 24 hours, the venular spasm is reduced, but the arteriolar spasm remains. No patent small vessels can then be observed in the injured area.[1]

The arteriolar and arterial spasm and the loss of intravascular fluids seen early in the frostbite syndrome can only partly account for the stasis seen in the capillaries; there is no entirely satisfactory explanation for the complete cessation of blood flow in the small vessels. Nevertheless, it might be anticipated that relieving the arterial spasm in the tissue just proximal to the frostbitten area might have some beneficial effects on the blood supply to the anoxic tissues beyond, especially if accomplished before irreversibility and thrombosis occur.[4]

This seemed to be the case under the conditions of our experiments. With the denervation of vascular structures in the injured extremity by sympathectomy immediately after freezing and thawing, there was significant increase in tissue survival. If as little as 24 hours elapsed before the sympathectomy was performed, however, irreversible damage had already taken place. Whether these irreversible changes involved the conversion of stasis into thrombosis, whether anoxic damage had already occurred, or whether sympathetic deprivation offered some direct salutory effect is not known at this time.

The experiments of Ederstrom *et al.*[8] indicate that in the dog foot the increased sensitivity of denervated vessels to circulating vasoconstrictor material[3] apparently develops as early as the first day after sympathectomy. By the third day it is manifested in the deeply anesthetized animal as a decreased flow in the operated foot compared to that of the control. Such a condition could explain the therapeutic ineffectiveness of sympathectomy three days before freezing. It might also explain the paradoxical observations that three-day sympathectomized feet froze more quickly than the controls. Immersion in the cold bath probably stimulates epinephrine release and thereby may cause vasoconstriction in the supersensitive vessels. Control vessels, however, are not only unsensitized, but are, on the contrary, subject to vasodilator influences of the deep anesthesia and

possibly of reflex protective mechanisms against the local effects of cold.[12]

The phenomenon of supercooling has been observed by many authors,[6, 23, 24] but none has been able to offer satisfactory explanation of why it happens. Conditions can be made less favorable for the formation of a nidus of ice crystal and thereby increase the incidence of supercooling, or the temperature might be altered to enhance supercooling,[24] but we are unaware of a physical explanation of why supercooling occurs. It might be mentioned that this phenomenon occurs also in the freezing of electrolyte and pure liquid solutions[6] and has been demonstrated by us in dead as well as living animals.

The increased damage observed in the supercooled rabbit feet may have been caused by the addition of a cold immersion type of stress to that of the standard freezing injury. Supercooling itself, according to Lewis's observations,[23] usually did not produce cold injury, but occasionally, if the exposure was long and the temperatures very low, wheeling and flare were seen.

Conclusions

The best treatment for freezing injuries of the extremities which is now available is based on rapid rewarming in warm water, but this regimen must still be improved. The experiments on rabbits presented here imply that increased tissue survival results from sympathetic denervation, but only if it is performed soon enough after the freezing injury. It has been noted, however, that in order to achieve maximum effectiveness (and possibly to be effective at all), it is necessary to deprive the frostbitten part of its sympathetic innervation with as little delay as possible—preferably immediately after thawing, and, in this experimental situation, within 24 hours.

The procedure of sympathetic denervation immediately following rapid thawing now awaits well controlled clinical evaluation.

Acknowledgment

The authors wish to thank R. I. Ramsbottom, HM1, USN, for his invaluable technical assistance and Anne B. Snodgress for her expert preparation of the histological specimens necessary for the experimental work described herein.

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