STUDIES ON GANGRENE FOLLOWING COLD INJURY. I. A METHOD FOR PRODUCING GANGRENE BY MEANS OF CONTROLLED INJURY BY COLD[1]

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INTRODUCTION

The incidence of gangrene associated with a wide variety of injuries, in which infection is not a primary factor, continues to present problems of treatment at all levels of handling military casualties from the front line dressing stations to the large general hospitals. Gangrene results from injury due to freezing, prolonged exposure to wet and cold without freezing (immersion or trench foot), burns and vascular occlusion. It seems probable that the fundamental factors responsible for the appearance of gangrene are similar in all of these cases. A more detailed examination than has previously been made of the primary mechanisms by which gangrene is produced, and the steps which may be taken to prevent its occurrence, may have wide application to the general problem of the prevention and treatment of gangrene, irrespective of the primary injury.

Local injury resulting from exposure to cold may take one of several different forms depending chiefly upon the temperature to which the part is exposed. Injury occurring after relatively brief exposure to severe cold, in which the skin and sometimes deeper tissues are actually frozen, will be referred to as "frostbite" (1). It is this type of injury with which this series of papers is primarily concerned. Exposure to milder degrees of cold, especially for long periods of time and under conditions favoring rapid transfer of heat from the body, results in a type of cold injury in which no actual freezing of tissue occurs. This "trench foot" or "immersion foot" type of injury is not specifically considered in this study, although it is recognized that the fundamental features of both may be similar.

[1] The work described in this paper was done under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Stanford University.

The present investigation is an attempt to analyze the factors responsible for the production of gangrene following freezing (frostbite) and to examine methods of treatment of frostbite which are designed to prevent the appearance of gangrene or to reduce its extent. We have not been directly concerned with the surgical management of gangrene following cold injury or with nerve and vascular abnormalities which persist after the acute stages following injury by cold.

SUMMARY OF AVAILABLE PROCEDURES FOR PRODUCING EXPERIMENTAL FROSTBITE

Before it is possible to conduct an adequate study of methods for the treatment of gangrene following experimental frostbite, it is necessary first to obtain reproducible degrees of injury in experimental animals. A variety of methods have been used by previous workers for producing frostbite injury. Since in many cases the results are, to a large degree, dependent upon the method used for producing the frostbite, the available methods will be reviewed briefly.

1. *Liquid immersion.* Some of the first observations on the pathology of frostbite injury, made by Cohnheim (2), were carried out on rabbits' ears frozen by immersion in cold liquids. Lake (3) used liquid immersion in a study of experimental frostbite. Fell and Hanselman (4) produced shock in dogs by freezing the legs in CO_2 and alcohol. Recently Lange and Boyd (5) produced frostbite by liquid immersion, but the limb was first inserted into a boot of thin rubber to protect it from wetting. These authors refer to this procedure as "contact cooling."

2. *Sprays.* In early work Rischpler (6) and others (7) utilized ether spray for producing cold injury. Sprays of ethyl chloride have been used extensively by the Russian investigators Ariev (8, 9, 10) and Orlov (11).

3. *Cold air.* Exposure of a limb to moving cold air most nearly approximates the conditions under which frostbite of face, ears, and hands occurs. Ariev (12) and Ariev and Esberg (9) have reported results obtained by the use of this method. Greene (13) obtained a "stand-

ard frostbite" of mouse tails by the use of a small chamber containing the tail, surrounded by a larger chamber filled with solid carbon dioxide.

4. *Direct contact with cold solids.* Precooled metal bars were applied to the skin by Lewis and Love (14) for the production of cold injury. The direct application of solid carbon dioxide to the skin was used by Harkins and Harmon (15) and Tittel (16) and beakers containing solid carbon dioxide were used by Lange and Boyd (5).

The method selected for the production of quantitative frostbite should be one which is applicable to fingers and toes, since injuries in man most often involve these parts (12, 7). Uniform cooling of all surfaces of these parts is difficult by means of the direct application of cold solids. The spraying of volatile liquids upon the skin of appendages is also difficult to control in extent and might be expected to be much more variable in effect than cold air or liquid applied directly. While it is true, as Ariev points out, that cold air is most nearly comparable to the conditions under which frostbite sometimes occurs in man, it does not seem so suitable as liquid immersion for the purposes of producing a quantitative frostbite in experimental animals. The cooling effect of the air is dependent not only upon the temperature, but also upon the air velocity, thus introducing an additional variable. Very long exposures to cold air are necessary to produce frostbite if the velocity is low. Ariev (12) found that after exposing rabbits' ears to still air at a temperature of − 40° C., the ears were still at a temperature of + 11° C. after 4 hours.

The method which we have selected for the production of experimental frostbite involves the immersion of an extremity in a liquid mixture cooled to the desired temperature. Frostbite of rabbits' feet and ears has been compared using varied temperatures from − 10° C. to − 55° C. for a constant time, and using varied times of exposure at − 55° C. Frostbitten extremities in which the injury was produced by exposure to cold air have been compared to those in which the injury was produced by immersion.

Conditions for the production of quantitative frostbite

Rabbits' feet and ears were frozen by immersion in a liquid mixture consisting of 50 per cent by volume ethylene glycol in water, to which were added 150 ml. per liter of 95 per cent ethyl alcohol. This mixture was cooled as needed by the addition of solid carbon dioxide. A mixture of this composition is suitable for cooling to about − 55° C., at which temperature it is still fluid. Lower temperatures may be achieved by the addition of larger amounts of alcohol and CO_2. The relatively high specific heat of this liquid makes it preferable to ether or acetone which have been used by other workers.

Rabbits were anesthetized by the administration intraperitoneally of dial, 90 mgm. per kgm. Occasional experiments were done using pentobarbital (37.5 mgm. per kgm.) anesthesia. The hair was removed from the area to be frostbitten by close clipping (Oster small animal clipper, size 40 blades). Immediately before frostbite the barbiturate anesthesia was supplemented with ether to the point of disappearance of the corneal reflex. This level of anesthesia was found to be necessary to prevent struggling during the period when freezing of the tissue occurred. Hind feet of rabbits were immersed in the freezing mixture as far as the tuberosity at the base of the fifth metatarsal. The distal 4 to 5 cm. of rabbits' ears were frozen in a similar manner. During the period of immersion the ear or foot was kept in motion in order to equalize the temperature of the liquid immediately surrounding it.

Frostbite was produced in a few animals under barbiturate anesthesia only, and in one with local anesthesia (procaine infiltration). The course following injury was not different from that occurring after barbiturate-ether anesthesia.

The ethylene glycol-alcohol mixture was tested for possible irritant properties by immersing rabbits' feet and ears in it at room temperature for periods as long as 30 minutes. These immersions were not followed by hyperemia or by visible alterations of any kind from the time of removal of the part from the mixture to the end of the observation period 3 weeks later.

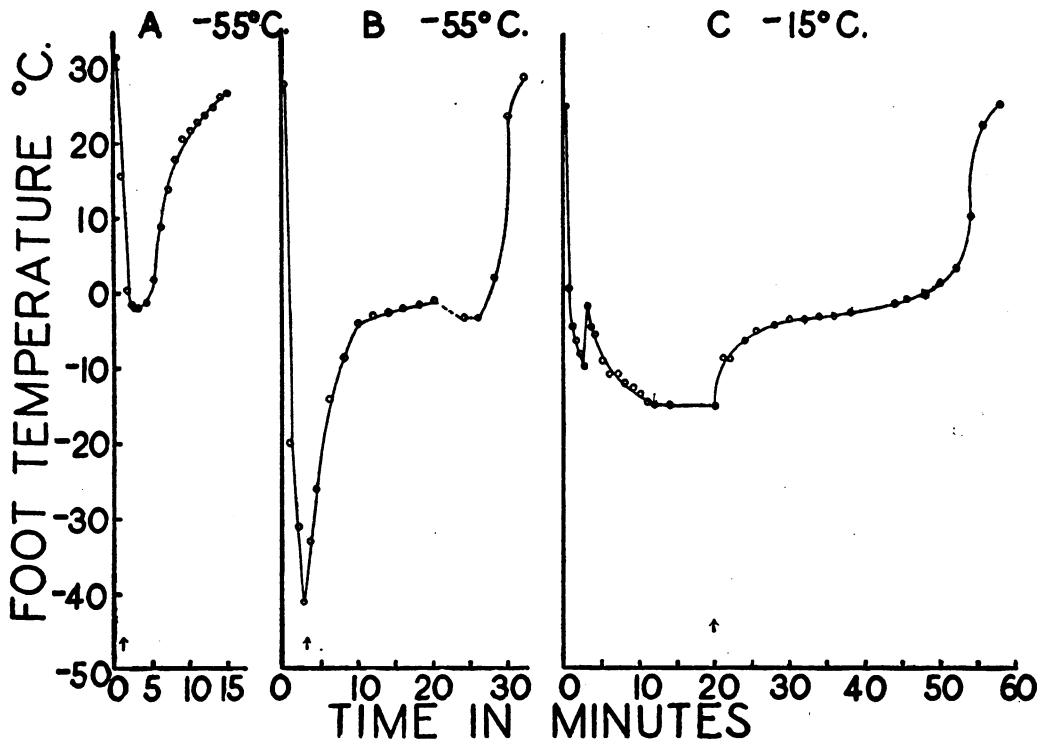
For comparison with the results obtained by immersion in cold liquids, a few rabbits' feet and ears were frostbitten by exposure to a blast of cold air at approximately − 68° C. Compressed air at approximately 18 pounds per square inch pressure was dried, cooled by passing through a chamber filled with solid carbon dioxide, and then directed into a second chamber of about 1 liter capacity which contained the foot or ear to be frozen.

The temperatures of the liquid mixtures and cooled air were measured with Weston bi-metallic thermometers which were checked at intervals by comparison with mercury thermometers.

Deep tissue temperatures in rabbits' feet during immersion in freezing liquid

In order to determine the extent of temperature reduction during frostbite, thermocouples were implanted in the feet of anesthetized rabbits and the temperature determined during immersion in the freezing liquid and during the thawing period after removal from the cold liquid.

A thermocouple made of No. 26 gauge iron-constantan wire was inserted into the foot above the level of immersion and thrust distally deep into the foot at the base of the toes. Temperatures were determined by means of a Leeds and Northrup type K potentiometer. Temperature measurements were made during three different types of frostbite: (a) 1-minute immersion at − 55° C., (b) 3-minute immersion at − 55° C., and (c) 20-minute immersion at − 15° C. The results of representative experiments are given in Figure 1.



DEEP TISSUE TEMPERATURE CHANGES IN RABBITS' FEET DURING FREEZING AND THAWING

Temperature measurements made by means of thermocouples implanted deep in the foot near the base of the toes. See text for complete description. In each case the foot was immersed in the freezing liquid at 0 time and withdrawn at the time indicated by the arrow.

FIG. 1–A. 1-minute immersion at − 55° C.

FIG. 1–B. 3-minute immersion at − 55° C. Thermocouple moved slightly in foot at point indicated by dotted line.

FIG. 1–C. 20-minute immersion at − 15° C. The break in the curve at 3 minutes represents temperature changes resulting from supercooling (see text).

During 1-minute immersion at − 55° C. the deep tissue temperature falls to approximately zero (Figure 1–A). Removal from the liquid is followed by a slight additional fall in temperature to about − 2° C. Upon removal the foot is stiff and the superficial tissues are solidly frozen. The foot remains frozen (with deep temperature at 0° or below) for about 5 minutes, and then warms until it reaches 27° C. after about 15 minutes.

During 3-minute immersion at − 55° C. the lowest temperature reached by the deep tissues was − 40° C. (Figure 1–B). After removal from the freezing mixture the foot warmed slowly until blood flow was reestablished 27 minutes after immersion. Rapid warming of the foot by the blood then raised the temperature to about 30° C. within the next 5 minutes.

During immersion at − 15° C. the foot froze after about 3 minutes and reached temperature equilibrium with the bath in 12 minutes (Figure 1–C). The phenomenon of supercooling, which has been observed frequently in biological material (17), is well illustrated in Figure 1–C. The foot cooled to − 9.4° C. in 155 seconds without freezing. During the next 28 seconds the temperature increased to − 1.5° C. and freezing occurred. The frozen foot then cooled to the temperature of the liquid. After removal from the freezing mixture the foot temperature remained below zero for 28 minutes and then increased rapidly.

EXPERIMENTAL RESULTS

Incidence of gangrene and extent of tissue loss following untreated cold injury

The general course of events leading to gangrene in frostbitten ears and feet will be considered in a subsequent paper. The data given here illustrate the uniformity with which gangrene is produced by controlled cold injury and serve as the basis for evaluation of the methods of treatment to be discussed later.

1. *Frostbite of rabbit ears*

Following immersion of the ear for time periods ranging from 60 seconds to 10 minutes, at temperatures from − 32° C. to − 70° C., the entire frostbitten portion of the ear became gangrenous and was eventually lost. Shorter periods of immersion at − 55° C. led to loss of varying amounts of the distal part of the ear.

In two animals the ears were frostbitten by means of an air blast at a temperature of − 68° C. In one case, after a 2-minute exposure, the ear became edematous, but no loss of tissue occurred. In the other, after 4-minute exposure, complete loss of the injured region resulted.

TABLE I

Incidence of gangrene and extent of tissue loss following frostbite of rabbit ears

A. Liquid immersion

Duration of immersion	Temp.	No. of animals	Per cent of animals developing gangrene on days after injury					Extent of tissue loss
			Wet stage		Dry stage			
			3 days	4 days	4 days	5 days	6 days	
min.	° *C.*		*per cent*		*per cent*			
10	−32	1	—	—	—	—	—	Complete[1]
3	−40	1	—	—	—	—	—	Complete
2	−42	2	—	—	—	—	—	Complete
2	−53	2	100	0	0	100	0	Complete
1½	−55	24	71	29	17	72	11	Complete
1	−55	11	80	20	20	80	0	Complete
1	−70	1	—	—	—	—	—	Complete
½	−55	1	0	100	0	100	0	80 per cent of injured region
¼	−55	1	100	0	0	100	0	80 per cent of injured region

B. Air blast

2	−68	1	No gangrene					None
4	−68	1	100	0	0	0	100	Complete

[1] Complete loss indicates loss of entire injured part of ear as far proximally as the line to which it was immersed.

TABLE II

Extent of tissue loss occurring after frostbite of rabbit feet

Duration of immersion	Temp.	Number of animals	Extent of tissue loss
min.	° *C.*		
Series 1			
1	−55	12	No tissue loss (11).[1] Distal phalanx of each toe (1).
2	−55	5	All toes (4). Complete except plantar pad (1).
3	−55	16	Complete to line of immersion (11). Complete except plantar pad (4).
Series 2			
3	−45	2	Complete to line of immersion (2).
3	−35	2	Complete except plantar pad (2).
3	−25	2	Distal and middle phalanges ot each toe (2)
3	−15	2	Foot did not freeze: no tissue loss (2).
Series 3			
15	−15	1	No tissue loss (1).
30	−15	1	No tissue loss; toe pads thickened (1).
60	−15	5	Complete to line of immersion (2). Complete except plantar pad (2). No tissue loss: foot did not freeze (1).

[1] Numbers in parentheses show number of animals.

The data showing the extent of tissue loss together with the time after exposure at which gangrene appeared are given in Table I. The injuries selected for the study of methods of treatment were (a) 1½-minute immersion at − 55° C., and (b) 1-minute immersion at − 55° C. The extent of tissue loss and the extent and rate of development of gangrene following these injuries were quite uniform, as may be seen from Table I. In every instance the whole of the distal portion of the ear to the point of immersion became gangrenous and separation occurred at the line to which the ear had been immersed. Following 1½-minute exposure at − 55° C., wet gangrene appeared in about 70 per cent of the animals on the third day after immersion and drying occurred on the fifth day. Following 1-minute exposure at − 55° C., 80 per cent of the animals developed wet gangrene on the third day and dry gangrene on the fifth day. The time of separation of the injured part of the ear varied somewhat depending upon the activity of the animal in the cage.

2. *Frostbite of rabbit feet*

The extent of tissue loss and the incidence of gangrene of rabbit feet following frostbite were determined in three series of experiments (Tables II and III).

TABLE III

Development of gangrene following frostbite of rabbits' feet

Duration of immersion	Temp.	No. of animals	Per cent of animals developing gangrene on days after injury					
			Wet stage		Dry stage			
			2 days	3 days	4 days	5 days	6 days	7 days
min.	°C		*per cent*		*per cent*			
1	−55	12	None		None			
2	−55	5	100	0	40	60	0	0
3	−55	15	60	40	20	60	13	7
3	−45	2	100	0	0	50	50	0
3	−35	2	50	50	0	50	0	50
60	−15	4	0	100	0	0	25	75

Series 1 consisted of animals in which one hind foot was immersed for 1, 2 or 3 minutes at a temperature of − 55° C. Following immersion for *1 minute* no loss of tissue occurred in 92 per cent of the animals, and gangrene did not develop in spite of massive edema. After 4 to 7 days the only consequences of the frostbite were thickening of the toe pads and slight to moderate induration of the foot. Following immersion for *2 minutes* gangrene appeared on the toes after the times given in Table III. Spontaneous amputation of the toes took place at varying times after the initial injury. The foot showed some scarring on the dorsum near the base of the toes and sometimes along the edges of the foot extending 0.5 to 1 cm. proximally. The stump was thickened, especially at the distal margin. Immersion at − 55° C. for *3 minutes* resulted in complete loss of the foot as far proximally as the line to which it was immersed in 69 per cent of the cases, and loss of the entire foot with the exception of a narrow tongue of tissue on the plantar surface in 25 per cent of the animals. The incidence of gangrene is given in Table III. Separation near the line of immersion occurred spontaneously; in some cases separation occurred 1 to 2 cm. distal to the line of immersion leaving a dry stump which was then worn away by the movements of the animal.

Series 2 consisted of animals in which one hind foot was immersed for a constant time (3 minutes) at − 45°, − 35°, − 25° and − 15° C. The extent of tissue loss is given in Table II and the incidence of gangrene in Table III. Freezing of the foot did not occur at − 15° C. in 3 minutes. At the other temperatures gangrene developed and varying amounts of tissue were lost, the injury being more severe at the lower temperature.

Series 3 consisted of animals in which one hind foot was immersed for longer periods of time, up to 1 hour, at − 15° C. Freezing of the foot usually occurred after 10 to 15 minutes at this temperature. In one animal, given a supplementary dose of pentobarbital immediately before frostbite and requiring artificial respiration, the foot did not freeze during 1 hour at − 15° C. When freezing did not occur edema was absent and gangrene did not develop. Following immersion of 60 minutes at − 15° C. the final tissue loss involved the entire foot in 2 cases and all except a narrow segment of the plantar surface in two others. The foot retained a flattened shape, with toes spread, for several weeks, and induration was severe. Gangrene developed somewhat later in these animals than in those injured at lower temperatures (Table III).

The animals comprising Series 1 and those in Series 3 injured by immersion for 60 minutes at − 15° C. serve as controls for evaluation of methods of treatment of experimental cold injury to be considered in a subsequent paper.

Effect of precooling on the extent of tissue loss following frostbite of rabbit feet

In three animals the foot was immersed in the liquid mixture at a temperature of 0° to + 2° C. for 30 minutes preceding a frostbite of 1-minute duration at − 55° C. In all instances the resulting injury was more severe than following a frostbite of only 1 minute at − 55° C. Parts of the toes of all three animals were lost; in one the loss involved one toe and the distal phalanx of another; in the second, only the distal phalanx of one toe; and in the third, the distal and middle phalanges of two toes. In contrast to these results, frostbite of 1 minute at − 55° C. without precooling usually resulted in no loss of tissues, but only thickening of the toe pads (Table II).

One animal was placed in a cold room at + 4° C. for 2 days preceding frostbite. The hair had been removed from both feet. Exposure of one foot for 1 minute at − 55° C. immediately upon removal from the cold room resulted only in the usual thickening of toe pads and no loss of tissue. The rectal temperature upon removal from the cold room was 38.7° C.

On one animal the hair was removed from both feet and the animal was placed in the cold room at + 4° C. for 24 hours. The animal was then anesthetized with dial and returned to the cold room for 2 hours, by which time the rectal temperature had fallen to 35° C. Following frostbite for 1 minute at − 55° C. the foot was slightly thickened, but no loss of tissue ensued.

SUMMARY

Controlled cold injury (frostbite) of rabbit ears and feet was produced by immersion of the depilated part in a water-ethylene glycol-alcohol mixture cooled to the desired temperature by means of the addition of solid carbon dioxide.

The immersion of ears of anesthetized rabbits in liquid at temperatures ranging from − 32° C. to − 70° C. for periods of 1 to 10 minutes resulted in freezing of the ear, edema, gangrene and spontaneous amputation of the entire injured part of the ear. Immersion for 15 or 30 seconds at − 55° C. resulted in loss of about 80 per cent of the injured part of the ear.

The effects of immersion of the hind feet of anesthetized rabbits in cold liquid varying from − 15° C. to − 55° C. for different lengths of time were determined. At − 55° C. the extent of tissue loss could be increased by increasing the duration of exposure from 1 to 4 minutes. At − 15° C. the deep tissue temperature of a foot became equal to that of the bath in 15 minutes. At this temperature the extent of tissue loss could also be increased by increasing the duration of exposure. When the time of exposure was maintained constant at 3 minutes and the bath temperature varied from − 15° C. to − 45° C., progressively greater loss of tissue was encountered with reduction of exposure temperature.

Precooling of feet for 30 minutes at about + 2° C. increased the severity of injury following a subsequent exposure for 1 minute at − 55° C.

Gangrene did not result from exposure for 1 hour at − 15° C. if freezing of the tissue did not occur. Gangrene resulting from cold injury was qualitatively identical in each case regardless of the time or temperature of exposure.

Readily reproducible degrees of injury and amounts of tissue loss could be obtained by exposure at a given temperature for a given period of time.

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