Activity in Mammalian Peripheral Nerves during Supercooling

Abstract. *Excised peripheral nerves from several species of mammals from interior Alaska continue to operate when supercooled to temperatures as low as −6°C. In some nerves, spontaneous freezing begins before activity ceases. If the nerves are not allowed to remain in a partially or completely frozen state for a prolonged period they will regain their activity when rewarmed.*

The freezing point of serum for a number of mammals lies between −0.55°C and −0.62°C, and the freezing point of cerebrospinal fluid in man is −0.57°C (*1*). With some allowance for variability of tissue composition it seems safe to presume that the freezing point of nerve tissue will not be lower than −1.0°C. For the purposes of this report any whole nerve existing in the completely unfrozen state at temperatures lower than −1.0°C will be considered supercooled.

The ability of nerves of poikilothermic animals to conduct when supercooled has been clearly demonstrated by a number of studies, but it is a common belief that conduction in mammalian nerves usually ceases in the region of 5° to 15°C. Studies conducted on hibernating species have indicated that peripheral nerves of these mammals function at lower temperatures than the nerves of nonhibernators (*2*). In addition, nerves from poorly insulated appendages such as the rat's tail will conduct at temperatures approaching 0°C (*3*).

For several years we have been examining the function of mammalian peripheral nerves over the temperature ranges at which they are active, looking in particular for evidence of cold acclimatization. During a comparative study concerned with temperature relations of the ventral caudal nerves of muskrats from a cold climate (interior Alaska) and a warm climate (southern Louisiana), it was noted that the excised nerves continued to function very well at 5°C. Provisions were accordingly made for cooling the nerves, inside a moist chamber, in a mixture of salt and ice that provided temperatures to −15°C. With this method of cooling, the nerves remained excitable and would conduct action potentials at temperatures as low as −4.5°C. The ability to conduct at such temperatures was observed consistently in the nerves of muskrats from both Alaska and Louisiana.

Finer control of temperature and greater flexibility in stimulation and recording were obtained with a metal nerve chamber that provided temperatures to −30°C with an accuracy of ± 0.3°C. We have since examined the effect of temperature on activity of nerves obtained from a variety of mammals found in the vicinity of Fairbanks, Alaska (65°N). The species studied were muskrat (*Ondatra zibethica*), beaver (*Castor canadensis*), red fox (*Vulpes fulva*), red squirrel (*Tamiasciurus hudsonicus*), coyote (*Canis latrans*), porcupine (*Erethizon dorsatum*), marten (*Martes americana*), and mink (*Mustela vison*).

Median segments of both caudal and tibial nerves were tested wherever possible. Only the characteristics of larger, rapidly conducting fibers are reported. For all animals studied, conduction velocities for these fibers fell within the range 25 to 74 m/sec at a nerve temperature of 35°C. Nerve temperatures were measured with three fine thermocouples placed adjacent to different sections of the nerve. Except when rapid temperature changes occurred, such as during freezing, temperatures were found to be accurate to ±0.2°C. Of eight species tested, the caudal nerves of five, including muskrat, beaver, mink, red squirrel, and marten, were consistently found to conduct in the temperature range −1° to −6.0°C (Table 1). Of the tibial nerves tested, only those from beaver were consistently able to conduct impulses in this temperature region.

In many instances, spontaneous rewarming occurred when the nerves reached temperatures of −2° to −6°C; this was apparently due to the evolution of heat during freezing of the supercooled nerves. The occurrence of spontaneous rewarming was not correlated with the speed at which the nerves were cooled (2°C/min to 0.1°C/min). Figure 1 shows a typical record in which spontaneous rewarming (SR) began at −6.0°C. The air temperature in the chamber adjacent to the nerve increased about 3.3°C in less than 30 seconds. A portion of this increase may have been contributed by the freezing of a small amount (less than 0.15 ml) of Locke's solution adhering to the floor and walls of the chamber. Tests

Table 1. Characteristics of caudal and tibial nerves at low temperatures. Mean values are shown with ranges in parentheses.

Species	*N*	Lowest temperature at which nerve activity was present (°C)		No. of caudal nerves freezing	Temp. of caudal nerves at onset of freezing (°C)
		Caudal nerve	Tibial nerve		
Muskrat	15	−4.8 (−5.5 to −3.5)	1.9 (−1.3 to +3.1)	6	−4.6 (−5.1 to −4.0)
Beaver	11	−5.0 (5.6 to −4.0)	−0.7 (−3.8 to +3.8)	5	−5.2 (−5.6 to −4.6)
Mink	1	−5.1		1?	
Marten	2	−4.7 (−5.0 to −4.3)	2.0	1	−5.0
Red squirrel	9	−3.8 (−6.0 to +3.1)		6	−5.3 (−6.0 to −4.5)

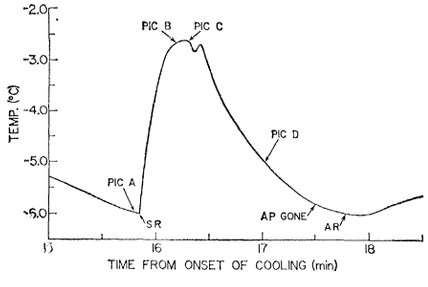

Fig. 1. Temperature curve for caudal nerve of red squirrel, showing spontaneous rewarming. *SR*, Onset of spontaneous rewarming. *AP gone*, Point at which action potential could no longer be elicited; *AR*, artificial warming initiated; *Pic A*, *B*, *C*, and *D* are points at which exposures were made for the action potentials shown in Fig. 2.

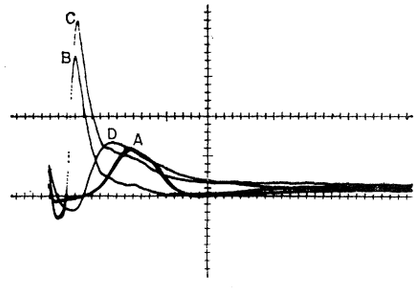

Fig. 2. Multiple exposure showing increase in height, conduction velocity, and changes in waveform of action potential during spontaneous rewarming. *A*, Just before spontaneous rewarming; *B* and *C*, at height of spontaneous rewarming; *D*, near end of spontaneous rewarming. Oscilloscope sweep, 20 msec/cm; sensitivity, 1 mv/cm; nerve stimulus supermaximal.

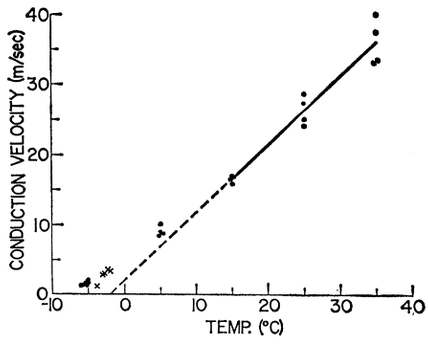

Fig. 3. Conduction velocity in red squirrel caudal nerves (fast fibers) plotted as a function of temperature. Filled circles are values obtained prior to spontaneous rewarming. Crosses are values during spontaneous rewarming.

showed that freezing of Locke's solution with concomitant warming of chamber air did not occur between 0° and −10°C without a nerve in the chamber.

That the temperature rise associated with spontaneous rewarming occurred within the nerve is shown by the large increases in the height of the action potential and in the conduction velocity that occurred at the onset of rewarming (Fig. 2, *B* and *C*). The studies of Mazur (*4*) on the thermodynamics of intracellular freezing predict that at low rates of cooling, such as those used in our studies, intracellular freezing would be unlikely at tissue temperatures warmer than −10°C. It is therefore probable that the spontaneous rewarming was associated with the freezing of extracellular fluid. The action potential usually disappeared soon after the temperature rise associated with spontaneous rewarming began its decline, lending support to the idea that the nerves were progressively freezing at this point. Nerves that were gently touched immediately after the disappearance of the action potential were stiff and opaque.

When nerves were cooled to the temperature where the action potential disappeared, or where spontaneous rewarming occurred, and then warmed again to 25°C, full recovery of function usually occurred. If artificial rewarming was not begun soon after disappearance of the action potential, the nerves either failed to recover or showed functional changes. In several instances cooling was continued after the disappearance of excitability, but none of the nerves cooled below −7°C recovered their excitability when rewarmed to 25°C.

The temperatures at which spontaneous freezing occurred are in the same range that Smith and her coworkers reported for spontaneous freezing in supercooled hamsters (*5*). Failure of excised nerves to recover function following freezing at −4° to −7°C occurred in the temperature range from which Smith (*5*) and Popovic (*6*) reported deaths from freezing of whole mammals. Our results with supercooled nerves also agree well with the recent findings of Luyet and Gonzalez (*7*) that whole muscle in rats can survive freezing for 15 to 20 minutes at −5°C, and not at all at −10°C. Evidence for recovery from freezing of peripheral nerves in man was provided recently by the observation of Mills (*8*) that frozen limbs may show a return of cutaneous sensitivity after being thawed by rapid rewarming techniques. Our results would corroborate such recoveries if nerve temperatures never fell below −4° to −7°C.

Figure 3 shows a plot of conduction velocity as a function of temperature in the caudal nerve of a red squirrel. Determinations of conduction velocity just prior to or during spontaneous rewarming are plotted in the lower left corner (temperatures below 0°C). Conduction velocity is commonly reported to be a linear function of temperature. Such a linear relationship appears to hold for our data between 15° and 35°C, but values obtained at 5°C and during supercooling are somewhat higher than might be expected.

It is not known if peripheral nerves ever must function in the supercooled state in an intact animal. The fact that caudal nerves of muskrats from a warm climate conduct in the supercooled state shows that exposure to severe cold is not, at least in every case, associated with conduction capability at supercooled temperatures. Perhaps the most important feature common to those species exhibiting subzero nerve function is the rather wide temperature range over which the body regions supplied by such nerves normally operate.

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Ultrastructure of Vegetative and Reproductive Apices of Chenopodium album

Abstract. *The apical meristem of the vegetative shoot of* Chenopodium album *(lamb's-quarters) exhibits alterations in cytoplasmic structure as early as 3 hours after the plant has been subjected to one photoinductive cycle which promotes flowering. The endoplasmic reticulum shows an altered distribution and there is evidence of an increase in acid phosphatase production. Dictyosomes increase in number per cell by the end of the second inductive cycle.*

The volume of literature relative to the process of flowering is considerable and there are recent reviews of the subject (*1*). Interest has centered mainly around the areas of response of the plant to its environment, the site of synthesis and movement of a flowering substance, and biochemistry of the receptor pigment system. There are very few studies in which attempts were made to correlate strictly physiological processes with early morphological changes which occur at the reactive sites—the shoot apical meristems.

In most of the early morphological studies of flowering, no attempts were made to control flowering experimentally, and in many instances such control would have been virtually impossible. In more recent studies, certain short-day plants in which flowering can be controlled conveniently have been used, for example, *Xanthium pennsylvanicum* (cocklebur), *Chenopodium album* (lamb's-quarters), and *Pharbitis nil* (Japanese morning glory). Several of these studies have yielded data relative to qualitative changes in such substances as nucleic acids and total proteins (*2–4*). It has been well established that only an active bud can be in-