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MAMMALIAN PERIPHERAL NERVE FUNCTION AS RELATED TO TEMPERATURE.

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MAMMALIAN PERIPHERAL NERVE FUNCTION AS RELATED TO TEMPERATURE

A

DISSERTATION

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MAMMALIAN PERIPHERAL NERVE FUNCTION

AS RELATED TO TEMPERATURE

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ABSTRACT

Temperature relationships were investigated in several types of peripheral nerve in a variety of mammals from interior Alaska. Excised caudal nerves from animals with poorly insulated tails such as the muskrat and red squirrel showed lower conduction velocity slopes and lower action potential extinction temperatures than caudal nerves from species with well insulated tails. Alaska muskrat caudal nerves showed significantly lower conduction velocities than Louisiana muskrat caudal nerves at temperatures above 5°C.

Most tibial nerves showed a greater decrease in function with decreasing temperature when compared with caudal nerves. Phrenic nerve function was effected by low temperature to an even greater extent. Temperature relationships of refractory period and action potential duration in various nerves followed the general pattern seen for conduction velocity.

Conduction at supercooled temperatures was seen in the caudal nerves of five of eight species tested and was most prominent in animals with poorly insulated tails. Freezing of supercooled nerves, accompanied by spontaneous rewarming, often occurred between -3° and -6°C. Marked action potential changes were associated with spontaneous rewarming. Nerves recovered from freezing if artifically rewarmed soon after disappearance of the action potential.

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INTRODUCTION

Living tissue functions within a narrow temperature region, the upper limits of which are determined by the denaturation temperatures of proteins or the inactivation of critical enzymes, and the lower limits of which are determined by chemical inactivation or freezing. From the standpoint of time required for a given process and the integration of that process with overall body function, tissues operate optimally at temperatures most commonly provided in the living organism. In most mammals this optimal tissue temperature is maintained well above the freezing point by an insulating fur covering and other behavioral or physiological temperature-regulating mechanisms.

Mammals living in colder climates must have enough insulation to maintain body warmth in the cold, but in meeting this requirement they are faced with the problem of eliminating excess heat when the weather is warmer or when they are exercising strenuously. Some northern mammals have met the heat loss problem by making use of bare regions, usually located on or including their extremities, as heat radiators. In order that these special regions of heat transfer not allow an unnecessary or even dangerous amount of heat loss when the animal is in the cold, their temperature must be kept as low as possible, thereby slowing the rate

of heat transfer to the environment. Some examples of lightly-insulated body surfaces from which low temperatures have been measured (Irving and Krog, 1955) include the bare foot pads of dogs and porcupines, the hooves and legs of reindeer, and the bare tail of the muskrat (Johansen, 1962). Assuming that the temperature of such bare surfaces may also rise to nearly the level of deep body temperature, a fluctuation in tissue temperature of almost 40°C is possible.

The temperature fluctuations experienced by the extremities of many northern mammals must involve the nerves found in such regions. As a consequence of these changes in nerve temperature, a number of questions arise: What are the lowest temperatures at which mammalian nerves may function? Are there differences in temperature-related nerve function among various species of mammals living in a cold climate? Do the various peripheral nerves of a given animal that are exposed to different body temperature environments exhibit differences in temperature-related nerve function? Do differences in temperature-related nerve function occur in animals of the same species living in different climates? And, ultimately, how can messages which must be constant in nature, i.e., such as appendage position, be transmitted unchanged through nerves whose functional characteristics change with temperature?

With the exception of the last, least accessible problem, this dissertation attempts to answer, at least in part, the various questions noted above. To achieve this end, the relationships between temperature and peripheral nerve functions have been studied in a variety of Alaskan mammals over the past three years. Animals from the variety of environments were used, including semiaquatic, terrestrial, and arboreal forms. In addition, a group of muskrats from southern Louisiana has been compared with muskrats from interior Alaska with respect to temperature-related nerve activity.

In general, the methods used in analyzing nerve function with respect to temperature made use of standard electrophysiological recording techniques. As many electrical characteristics of the nerve as could be conveniently measured were included in the experimental procedure. For reasons that will be discussed in the METHODS section, all studies were made on excised nerves.

The rather extensive literature from earlier studies, most of which concerns frogs or other poikilotherms, is reviewed in the following section. Experimental techniques common to the various studies reported are described in the METHODS section. To attempt to lessen the confusion that might arise from the various questions considered, results

and discussion of different problems are considered under separate subheadings. An attempt is made to integrate the results of the various thesis findings in the section titled GENERAL DISCUSSION AND CONCLUSIONS.

Reports concerning portions of the work reported herein have recently been published (Irving, 1964a, 1964b, 1965; Miller, 1965, and Miller and Irving, 1965).

PREVIOUS WORK

Amphibia

The earliest studies concerned with the effects of temperature on peripheral nerve activity apparently date from the period immediately following the demonstration of the nerve action potential by du Bois-Reymond in 1848. Afanasieff (1865) attempted to resolve the conflicting temperature information obtained on frog nerve by von Eckhard, Harless, and Scheleske (all cited in Afanasieff, 1865). Afanasieff's remarkable excitability studies of frog sciatic nerve showed that in a nerve-muscle preparation cooling to -8°C did not completely destroy excitability. Clonic muscle spasms (which can best be attributed to freezing of the nerve) were seen between a nerve temperature of -4 and -8°C. A marked decrease in nerve excitability was noted following the muscle spasms, and the presence of any nerve function at that point seems very doubtful. The nerves recovered excitability if they were rewarmed within one hour following occurrence of the muscle spasms.

Shortly before the turn of the century, Gotch and Macdonald (1896) noted that excitability of frog sciatic nerve decreased with decreasing temperature. Stirling (1895) noted in his physiology text that cold could abolish or diminish excitability of frog nerve, but that it did not

affect conductivity. This statement is not surprising when one considers the fact that all of the earlier studies of nerve function, including those made during the early part of this century, used muscle response in a nerve-muscle preparation as the measure of nerve activity.

Boycott (1902) conducted a careful series of experiments on the effect of temperature on frog nerve, using a sciatic gastrocnemius preparation to observe the effects of localized (less than one centimeter) cooling of the nerve. Though apparently not aware of Afanasieff's earlier work, Boycott arrived at some of the same conclusions. He was able to definitely correlate the production of muscle tetanus during intense nerve cooling with actual freezing of the nerve. Contrary to Afanasieff, Boycott noted that, following freezing, "Both excitability and conductivity are as a rule permanently lost in the cooled area." The freezing tetanus usually occurred in the region of -7°C. No decrease in muscle response was noted when a region of the muscle nerve was cooled as long as the cooling was not allowed to proceed to the freezing point of the nerve. Boycott thus assumed that conductivity of the nerve was not effected by cold. Conduction velocity was not measured, however.

Several years later, Bühler (1905), aware of Afanasieff's work but apparently unaware of the studies of

Boycott, also studied the effect of (localized) low temperature on conductivity of frog sciatic nerves. The temperature at which conductivity suddenly stopped or decreased, called the "critical temperature," averaged -7°C. Bühler recognized the fact that the critical temperature corresponded to freezing of the supercooled nerve. Recovery of some nerves following (apparent) freezing was observed.

The effect of temperature on nerve excitability at different stimulus durations was studied within a limited temperature range (9° to 29°C) by Lapicque (1907). It was found that at short stimulus durations excitability decreased with decreasing temperature, but with long-lasting stimuli excitability at lower temperatures actually increased. Two distinct antagonistic phenomena were postulated as contributing to the excitation process.

Tait (1906, 1908a, 1908b) conducted a series of experiments on the influence of temperature on frog nerve (sciatic). Most of his observations confirmed previously known facts. In common with most of the earlier studies concerned with the effects of temperature on nerve function, a serious drawback to his experimental technique was the fact that only a very small section of nerve, that portion lying on a constricted tube of 2mm diameter, was affected by changing temperature. Tait noted recovery of many nerves

following freezing, but the apparent recovery can be explained by the fact that such a small length of nerve was frozen, that even if it were killed, an action potential could "jump" the killed area.

The resting potential of frog nerve was studied with respect to temperature by Verzar (1911). He noted that the cooled portion of a nerve became electrically negative (which could now be interpreted as a depolarization) with respect to the remaining portions and that the membrane potential reached a maximum at 20°C.

The temperature coefficient of the refractory period of nerve (probably frog sciatic) was investigated by Adrian (1914). A coefficient of about three was found for both absolute and relative refractory periods between 10° and 20°C. There was some tendency for the temperature coefficient to increase as temperature decreased. Amberson (1930) also studied the effect of temperature upon the absolute refractory period of frog nerve. He claimed more accurate control of temperature in his studies. His results closely confirmed previous values obtained by Adrian.

Bahrmans (1932) measured the change in resistance of frog sciatic with decreasing temperature. He found a sudden resistance increase of three to five times at -6°C, the temperature at which nerve conduction disappeared. His

results thus tend to confirm the idea that the freezing point of supercooled frog nerves is near -6°C.

In agreement with the findings of previous studies, Schriever (1932) observed a lowered rheobase at low temperatures but found an increased chronaxie. In other words, at lower temperatures and at a given excitation voltage above the electrical threshold, the stimulus current must flow for a longer time in order to excite the nerve. However, the minimal amount of current needed to excite, regardless of the length of time it flows, is less at lower temperatures. It is difficult to reconcile these two seemingly opposite changes.

An often-quoted paper concerning the effects of temperature on peripheral nerve activity is that of Gasser (1931). A major advantage of this study over previous nerve studies lay in the use of the cathode ray oscilloscope and other specialized techniques for nerve recording [developed previously by Gasser and Erlanger (1922)]. The temperature coefficients for conduction velocity, spike potential, and refractory period were not found to differ significantly from previous studies made in the temperature range between 10° and 30°C. However, Gasser did report a large increase in all temperature coefficients at lower temperature, i.e., 5°C. He states that, "The durations of the rising and falling

phases of the spike of the action potential are affected to the same extent by temperature change."

Utilizing recording techniques that allowed them to study single, medullated frog nerve fibers, Schoepfle and Erlanger (1941) found, in contradiction to the work of Gasser, that cold prolonged the descent of the spike much more than the ascent and increased, rather than decreased, the spike height. The lowest temperature studied was apparently 8°C.

A careful study of the occurrence of local cold block in frog nerve was made by Boyd and Ets (1934). By varying their techniques of cooling they were able to freeze short lengths (probably about two millimeters) of frog nerves either with or without supercooling. Freezing with supercooling was accomplished by coating the cooling rod and/or nerve with Vaseline to prevent the condensation of water with its subsequent freezing and "seeding" of the nerve to freeze. Supercooled nerves froze at an average temperature of -6°C, while the nerves protected from supercooling by a special technique froze at approximately -1°C. Many nerves which had been frozen at -6°C recovered their function when rewarmed if they were soaked for some time in Ringers solution. All nerves frozen at higher temperatures recovered. Such nerves were, however, more susceptible to future cold

block (i.e., if warmed and then recooled). Prior to the work of Boyd and Ets, Garten and Sulze (1913) had reported that in nerves of a tropical frog the cold-blocking temperature was lower for a short stretch of nerve than for the entire trunk. This finding was refuted by Boyd and Ets as being an artifact caused by changes taking place most rapidly near the cut ends of the nerve.

Tasaki and Fujita (1948) conducted temperature studies on isolated single fibers of a toad (species not given). The temperature coefficient of the conduction rate was constant at 1.8 over the temperature range used (5° to 20°C). The spike duration showed a much larger coefficient of 3 to 3.5, while the spike height had a very low temperature coefficient of 1.3. Although it was not mentioned, it is apparent from their records that the falling phase of the action potential was prolonged much more by low temperature than was the rising phase.

Tasaki (1949) later reported that the rheobase is not affected by temperature but that the minimum quantity of electricity needed to excite a nerve fiber is increased by cold. The second part of this statement appears to contradict the first. However, the "minimum quantity" apparently refers to the second, or test, shock of a conditioning-test shock pair, rather than to a simple stimulus voltage-duration test.

The time course of the subthreshold shock is retarded by cold.

Further studies of the toad single fiber preparation have been made by Shirakawa (1959). He found an even larger Q_{10} for spike duration than had Tasaki and Fujita, averaging 5.0. Average Q_{10} values for the rising phase and falling phase of the spike were 3.1 and 5.5, respectively, confirming the fact that cooling greatly prolongs the falling phase. Resistance of the myelin sheath was much reduced at lower temperature, and the negative after-potential was also reversibly abolished by cooling.

In a series of papers Laget (1956a, 1956b) and Laget and Guerin (1955) reported his observations on the effect of temperature on peripheral nerve function in the frog. Except for the introduction of a hazy term, "thermal hysteresis," as applied to the lag in changes in nerve function following temperature changes, his studies merely confirmed previous findings.

Nasonov and Suzkal'skaia (1956) reported an interesting comparison between summer and winter frogs with respect to nerve excitability at different temperatures. Nerves from summer frogs cooled from 20° to 5°C showed a decreased threshold to long lasting impulses and an increased threshold to short ones. Nerves from winter frogs cooled over the same range showed a decreased threshold to

both long and short stimuli. The failure to find an increased threshold with short pulses in the cooled nerves of winter frogs seems difficult to explain.

The effect of temperature and narcosis on frog nerve (species not mentioned) has been the subject of a recent study by Sjodin and Mullins (1958). In contrast to Lorente de No' (1947), who found that maximum spike amplitude occurred at about 10°C, Sjodin and Mullins obtained maximum spike amplitudes at 35°C, with a rapid drop above this temperature and a slowly-accelerating drop below. As will be mentioned later, considering spike amplitude as an absolute value may lead to serious error due to the complexity of changes in spike configuration that can occur with changing temperature. Surprisingly, Sjodin and Mullins found that their frog sciatic nerves did not conduct below 2°C, a temperature well above that reported by most authors for action potential extinction in frog nerve.

Other Poikilotherms

The effect of temperature upon nerve function of poikilotherms other than frogs and toads has received little attention, but a considerable variation exists in the poikilotherms thus far studied with respect to the temperature at which nerves cease to conduct in the cold.

The lizards Lacerta muralis and Lacerta agilis succumb to cold only at temperatures well below 0°C, so it may be assumed that some nerve functions exist below this temperature (Weigmann, 1929). Weigmann noted that the sciatic nerve of Alligator mississippiensis ceases conducting between 3.5° and 8.5°C. The giant axon of the squid (Loligo forbesi) will conduct to at least -1°C (Hodgkin and Katz, 1949). Cold block occurs in large motor fibers of the lobster at 4° to 5°C, while smaller, oscillatory fibers block at 1° to 3°C (Wright, 1958). Veprintsev and Antonov (1959) state that the giant axons of the earthworm are able to conduct down to -6.5°C.

The studies of Hodgkin and Katz (1949) on the squid giant axon are of particular interest because they involve single fibers and because the large size of the axon (0.5 to 0.7mm) allows recording of full or nearly full potentials from an inserted microelectrode. With the giant axon preparation, the resting potential was found to remain nearly constant between 2° and 20°C and to decrease slowly up to 39°C. In contrast, the action potential decreased in magnitude above 2°C and began to fall precipitously at about 36°C.

A decrease in temperature caused a greater increase in the duration of the action potential falling phase than in

the duration of the rising phase, the respective Q_{10} values between 10° and 20°C being 3.2 and 2.0. The positive phase of the action potential attained a maximum at approximately 25°C.

Recording from single lobster motor axons with external electrodes, Wright (1958) found that the action potential remained nearly constant with mild cooling, but below 10°C the action potential decreased markedly. The rheobase was slightly reduced for stimuli of longer duration but increased for short stimuli. Chronaxie increased with decreasing temperature. Conduction velocity was reduced by a factor of three to four with a temperature reduction of 20°C.

In the case of the earthworm giant axon, temperature coefficients for conduction velocity and duration of rising and falling phases were similar in sign and quantity to those of other poikilotherm nerves. Threshold values for stimulating pulses shorter than 0.1 milliseconds were approximately doubled for a 10°C drop in temperature but showed no change for pulses longer than 0.6 milliseconds.

Mammalian Nerve Studies

A common finding in previous temperature studies of mammalian peripheral nerve has been that conduction is blocked at much higher temperatures than is the case for

poikilotherm nerves. It should be emphasized that most mammalian nerve studies have utilized the readily available dog or cat, and that the use of these domesticated forms may give a one-sided view of mammalian nerve function.

Perhaps the earliest reported study of the effect of temperature on dog sciatic nerve was made by Grutzner (1878). He found that motor fibers were blocked at 6°C, while sensory fibers conducted to 1°C. The cardio-inhibitory fibers of the dog vagus also underwent cold block between 2° and 6°C. The latter finding was confirmed by Howell, et. al. (1894), who also noted that the rabbit vagus underwent cold block at temperatures as high as 15° to 20°C. Boycott (1902) later stated that the cardio-inhibitory fibers of the rabbit vagus blocked at temperatures below 10°C. Boycott also made observations on the effect of cooling the ulnar nerve in man. The actual nerve temperature was not measured; but with the elbow in a -17°C bath for what apparently was a short time, slight sensory impairment was noticed, and marked sensory phenomena referable to the little finger and back of the hand were obtained.

The conditions of survival of excised mammalian nerve trunks have been extensively studied by Forbes and Ray (1923). They were not primarily interested in temperature effects, but in the course of their studies they noted that

sciatic nerve function in dogs, cats, and rabbits was suspended below 8°C.

A further contribution to the knowledge of temperature effects on mammalian nerve was made by Gasser (1928). In a consideration of the relation of action potential shape to conduction velocity he noted that the phrenic nerve of a dog showed a marked drop in action potential amplitude during cooling from 37° to 19°C. In addition it was found that the product of the duration of the rising phase of the potential wave and the conduction velocity is constant during a change in temperature.

The most extensive analysis to date on the effect of temperature on mammalian peripheral nerve has been made by Lundberg (1948). The sixth and seventh lumbar roots of the cat were used for A fiber studies, while the splenic nerve of the cow was used for work on C fibers. Both the resting membrane potential and action potential were analyzed. The resting A fiber membrane potential was found to be maximal at body temperature, and warming or cooling from body temperature level resulted in depolarization of the membrane. The point of thermal equilibrium or maximum membrane potential of C fibers was between 20° and 30°C; higher temperatures effected the potential much more than lower temperatures.

Action potentials of A fibers could not be elicited at temperatures below 7°C and disappeared in some nerves at 15°C. Spike height was maximal at 30° to 35°C. The temperature at which C fiber action potentials disappeared was quite variable, ranging from 20° to -5°C. Maximum spike height was developed between 5° and 10°C.

The effect of potassium on the thermal sensitivity of A and C fibers was also studied by Lundberg. Excess potassium was found to shift the temperature of maximum resting potential upwards in both fiber types. Treatment of nerves with potassium-free Krebs solution lowered the thermal equilibrium point for the resting potential and lowered the temperature at which spike height was maximal. In the case of A fibers, the blocking temperature for conduction could be lowered to 0°C by soaking the nerve in potassium-free solution.

The differential effect of cold on nerve fibers of different size and function has been the subject of several other studies, mostly of the cat. Lundberg (1948) found that in cooling the saphenous nerve the slower conducting δ elevation disappeared before the α. Douglas and Malcolm (1955) confirmed this, noting further that the intermediate δ and β components failed in that order with cooling and before there was a significant change in

the α component. The nonmedullated or C fibers conducted to a lower temperature than any medullated fibers.

The order of susceptibility of vagal nerve fibers to cooling appears to be the reverse of that found for other nerves. Torrance and Whitteridge (1947) and Whitteridge (1951) noted that the most cold sensitive vagal fibers were the stretch fibers, which conducted at the highest velocity. Douglas and Malcolm failed to find any clear-cut differences among various vagal fibers with respect to cold sensitivity.

The ability of hibernating mammals to survive prolonged body temperatures just above the tissue freezing point has led several investigators to look for special nerve properties in such species. Attention was first directed toward excitable tissue function in hibernating mammals by Tait (1922), who found that a phrenic nerve-diaphragm preparation of the hedgehog or woodchuck would retain activity to "very low temperatures," apparently near freezing. Nerve function in hibernators received no further attention until 1948, when Chatfield, et. al. (1948), compared the effects of cooling on nerve conduction in the golden hamster (a hibernator) and the rat. Hamster tibial nerves conducted to an average temperature of 3.4°C, while rat tibial nerves ceased conducting at 9°C. A further comparison of nerve function in hibernating and non-hibernating

hamsters failed to disclose any differences in thermal sensitivity of nerves from the two groups of animals. It was, therefore, concluded that the ability of hamster nerves to conduct at low temperature was a true species difference, rather than a more short-term change in the nerve which occurred when the animal was exposed to cold.

Kehl and Morrison (1960) described differences in the temperature coefficients of sciatic nerves from hibernating thirteen-lined ground squirrels, as compared with nerves from animals of the same species not in hibernation. In general, hibernation seemed to be correlated with a decreased temperature coefficient of conduction velocity and excitability. This change was accompanied by a lowering of the temperature at which conduction velocity and excitability reached theoretical zero.

Tait's studies of the phrenic nerve-diaphragm preparation were extended by South (1961) with a comparison of hibernating and non-hibernating hamsters and laboratory rats. Diaphragm muscle strips were tested for excitability and contraction characteristics both with direct stimulation and by indirect stimulation via the phrenic nerve.

Only two of ten rat phrenic nerves remained excitable at 10°C, whereas all hamster nerves conducted at

5°C, the lowest temperature used. Nerves of hibernating and non-hibernating hamsters did not differ with respect to temperature-related excitability or conduction characteristics. Action potential amplitudes were reported without mention of possible errors involved in their interpretation.

Marked excitability differences were found between diaphragm muscle strips of rats and hamsters. The former were barely excitable at 5°C with direct stimulation, while both hibernating and non-hibernating hamster muscle strips were readily excitable using either direct or phrenic nerve stimulation.

The uninsulated metatarsal region of gulls living in cold climates is known to reach internal temperatures near 0°C (Irving and Krog, 1955). This fact was used by Chatfield, et. al. (1953) to demonstrate adaptation to cold in the tibial nerve of the gull. The tibial nerves obtained from the metatarsal region of the leg of cold-adapted gulls consistently conducted at lower temperatures than the same nerves from gulls adapted to heat. Nerves from warm-adapted gulls showed intermediate properties. In addition, nerves from the bare metatarsal leg region of all three groups were able to conduct at lower temperatures than were nerves from the feathered tibial region.

The caudal nerve of the albino rat has been reported to function at temperatures down to 0.3°C (Chatfield and Lyman, 1954). These authors found no alterations in caudal nerve function in a group of rats exposed to continuous cold (5°C) for 7 to 30 days. More recently, Miller and Irving (1963) reported that changes in rat caudal nerve function do occur if the animals are exposed t outdoor winter cold for a considerable length of time (months). In the latter case, conduction velocity and excitability were diminished at higher nerve temperatuess, and it appeared that, rather than showing an increased capacity to function in the cold following exposure to winter cold, the animals showed decreased ability to function at warmer temperatures.

Summary of Existing Literature

The majority of studies dealing with the effects of temperature on nerve has been concerned with amphibians. It is generally agreed that excised frog nerves will conduct in a supercooled state and cease conducting when they freeze at about -6°C. Some evidence exists for temperature adaptations in frog nerve, the nerves of both summer frogs and frogs living in tropical regions showing cessation of function at temperatures higher than nerves from winter frogs or frogs from cooler regions. Information on

temperature coefficients of conduction velocity is variable, but conduction is slowed as temperature decreases, often by a factor of two to three for each 10°C drop.

Excitability, especially to stimuli of short duration, decreases with decreasing temperature, while the refractory period increases in duration with a temperature drop. Information regarding changes in action potential magnitude are conflicting and difficult to assess.

A small amount of work concerned with temperature effects on other poikilotherm nerves indicates gross similarities with amphibian nerve. Oscillatory responses in crustacean nerves are more difficult to elicit at low temperatures.

Mammalian nerve fibers are reported to cease conduction at temperatures higher than those reported for poikilotherms. Except for one report of unmyelinated, or C fibers, conducting to -5°C, mammalian nerve fibers, including all A fibers, are reported to cease conducting above 0°C, frequently failing at temperatures as high as 10° to 15°C. Temperature coefficients for the various characteristics of nerve function appear to parallel closely the values reported for poikilotherm nerves.

Nerves of an obligate hibernator are reported to change functional capability during hibernation,

allowing the animal to survive and function at lower temperatures than could be tolerated when not in hibernation. Similar changes in nerve function at lower temperatures have been noted for caudal nerves of laboratory rats exposed to winter cold. In contrast to the findings for an obligate hibernator, hibernation in facultative hibernators (hamster) is not correlated with changes in peripheral nerve excitability or conduction. No information has been found to exist on temperature-related nerve activity in wild, non-hibernating mammals, particularly those living in cold regions.

METHODS

General

The initial plan of study was simply to compare function in nerves from peripheral regions of several mammals accustomed to living in the cold. Northern mammals exhibit a wide range in the amount of insulation of their appendages and in the degree of temperature variation which these appendages undergo. Of the various body appendages, the tail seemed to offer the best possibility for interspecific comparison of the effect of temperature on nerve function.

The various Alaskan canines, including the red fox and coyote, all have very dense underfur on the tail with an ample covering of long (approximately 8 to 9 cm.) guard hairs. Such tails are much less likely to undergo marked temperature changes than, say, the hairless tail of a muskrat or beaver, especially considering the fact that the latter two animals are frequently subjected to the severe cooling effect of near freezing water. Other non-aquatic mammals such as the arboreal red squirrel have rather lightly insulated tails that probably cannot be efficiently maintained near internal body temperature and that must, therefore, be allowed to cool. Animals such as

the mink and marten occupy a position intermediate between the bare-tailed species and the heavily insulated forms.

Comparison of temperature effects on caudal nerves of various species was aided by the fact that the tail is supplied by two pairs of nerves, dorsal and ventral, that are anatomically quite distinct and consistent in position from one species to the next. Because of their larger size and the fact that they were more easily identified and handled, the ventral nerves were used for all studies of caudal nerve function.

The ventral caudal nerves arise from the lower region of the cord, with the initial contribution usually from the first sacral cord segment, the largest contribution from the second sacral segment, and additions from the first caudal through the third caudal segments. According to Chatfield and Lyman (1954), the caudal nerves provide, at least in part, motor innervation to the tail muscles. Judging from the relatively small muscle mass in the tail itself and the rather large size of the nerves even in the midtail region, the nerves must also have important sensory components.

Ventral caudal nerves of eight species, including the muskrat (Ondatra zibethica), beaver (Castor canadensis), red fox (Vulpes fulva), red squirrel (Tamiasciurus

hudsonicus), coyote (Canis latrans), mink (Mustela vison), marten (Martes americana), and porcupine (Erethizon dorsatum), have been examined. Only the first four species were obtained in sufficient numbers to allow statistical comparison of nerve properties.

Other than the tail, the appendage most subject to cooling, at least from the standpoint of lack of insulative covering, is the lower hind limb. Again, the legs of semi-aquatic species such as the beaver and muskrat are probably subject to more severe cooling than the legs of most other species. The lower limbs of some of the ungulates such as the caribou and moose might also be expected to show marked temperature drops in cold air. This has been measured in the reindeer by Irving and Krog (1955), who found skin and deep temperatures in the tibial region to be as low as 9°C in -31°C air.

The tibial nerve was selected as the lower limb nerve most suitable for temperature studies, and its characteristics were examined in beaver, muskrat, red fox, coyote, and porcupine. A single moose tibial nerve was also obtained.

Later in the studies it was realized that a peripheral nerve accustomed to constant, internal body temperature would serve as a useful comparison to the more

thermolabile caudal and tibial nerves. The phrenic nerve seemed to offer by far the best comparison since it is exposed to deep body temperature in the thorax and is of very homogeneous fiber composition. It arises mainly from the level of the fourth cervical segment, with contributions from the third and fifth, and is largely motor (Truex, 1959). Only the beaver phrenic nerve was examined.

For several reasons all of the nerves were studied *in vitro* rather than *in vivo*. For one thing, *in vivo* studies require use of an anesthetic for both humane and practical reasons. The exact action on the nervous system of most general anesthetics is little known, and their deleterious effects on overall body physiology is frequently great, especially in long term anesthesia. Local anesthetics exert their effects on peripheral nerve and are ruled out in studies of normal nerve function. In addition, nerve dissection in a living animal is slower and more difficult due to bleeding, and greater care must be taken to avoid damage to larger blood vessels. Cooling of nerves *in vivo* is also more difficult and time consuming, and if good circulation is maintained near the nerve, as it should be for an *in vivo* study, local temperature gradients may be a problem. In order to obviate reflex responses, it is also necessary to

isolate, or at least cut centrally, the nerve being studied. Finally, the great majority of peripheral nerve studies made to date has been on excised nerves, and practically all of the studies concerned with the effects of temperature on nerve have utilized *in vitro* preparations. In particular, the work of Forbes and Ray (1923) has provided useful guide lines for estimating the viability of excised nerves.

With the exception of the group of Louisiana muskrats, all animals were obtained within a 150 mile radius of Fairbanks, Alaska (65° North Latitude). Most were captured alive using collapsible wire "walk-in" traps of a size suited to the particular animal. Several beaver were obtained with snares. Except for a number of muskrats classed as "indoor muskrats," most animals used in the studies were killed within one week following capture. This procedure was followed in order to eliminate possible temperature acclimation or marked deterioration due to captivity. All animals were active and appeared to be in reasonably good health at the time they were killed. Only adult animals (as judged by gross body weight and length) were studied. Sex was not considered to be a significant factor in the studies, and members of both sexes are usually represented. Animals in captivity were maintained

on a variety of diets best suited to the needs of the particular species. Several muskrats remained in good health for periods of up to two years on a diet of rat chow (Purina) supplemented once or twice a week with lettuce and carrots.

Experimental Procedure

Animals were killed with a sharp blow on the head, or in the larger species, with a shot in the head from a .22 caliber firearm. A few larger mammals were shot in the field. Nerve removal was begun as soon as possible, usually within a few minutes, and in no case did more than one hour elapse before removal was begun.

For removal of the caudal nerve a skin incision was made just lateral to the ventral midline of the tail, the skin then freed around most of the tail's circumference and pinned out. The caudal nerves are deeply imbedded in bundles of tendon, which necessitated removal of overlying and immediately-adjacent tendons. To prevent any possibility of drying, as soon as a portion of the nerve was exposed, it was moistened with Locke's solution, and a gauze pad saturated with Locke's was placed over it. A suitable length of nerve was dissected free using a specially sharpened dental tool and micro-dissecting

scissors. A glass hook was used to elevate the nerve when necessary.

A suture of number 50 nylon thread was tied at each end of the nerve, and the distance between these two threads measured to the nearest millimeter. The nerve was then cut several millimeters outside of each suture and placed in fresh Locke's solution saturated with 95 percent oxygen/5 percent carbon dioxide. After remaining in this solution for a period of from five minutes to several hours, depending on whether or not another nerve was removed from the same animal, the nerve was then transferred to the nerve chamber. In no case did nerve removal require more than three hours from time of death, and most were removed within one and one-half hours. Removal without damage was difficult in the red squirrel due to the small size of the nerve and its close attachment to the vertebrae in the intervertebral regions.

For removal of tibial nerves a posterior-median incision was made from the ankle joint to the knee, or to well above the knee in small animals. Dissection was accomplished in much the same manner as for caudal nerves, except that in larger animals the nerve was split so as to obtain a large branch or fiber bundle. A nine to eleven centimeter length of nerve was usually obtained.

Removal of the phrenic nerve from beaver was accomplished by opening the thorax just lateral to the sternum, retracting the ribs, and dissecting the nerve free from the pericardial sack and diaphragm. About seven to nine centimeters of nerve could be easily obtained.

It was found that after the technique of nerve removal was fairly well in hand, it was possible to study two different types of nerve from the same animal. In such cases the second nerve was removed before measurements were started on the first, and testing of both nerves was usually complete within eight hours after death. After nerve removal, the animals were weighed, sexed, and gross measurements of body and tail length taken.

Two different nerve chambers were used in the studies. The first, constructed of 1/8 inch plexiglas, is shown in Figure 1. It was used only for the initial comparison of Alaska and Louisiana muskrats. It was constructed only as a moist chamber, provision not being made for periodic flushing of the chamber with Locke's solution. For this reason a shallow layer (about two millimeters) of Locke's was left in the bottom of the chamber to help provide water vapor saturation of the chamber air. Temperature changes of the chamber air were made by changing

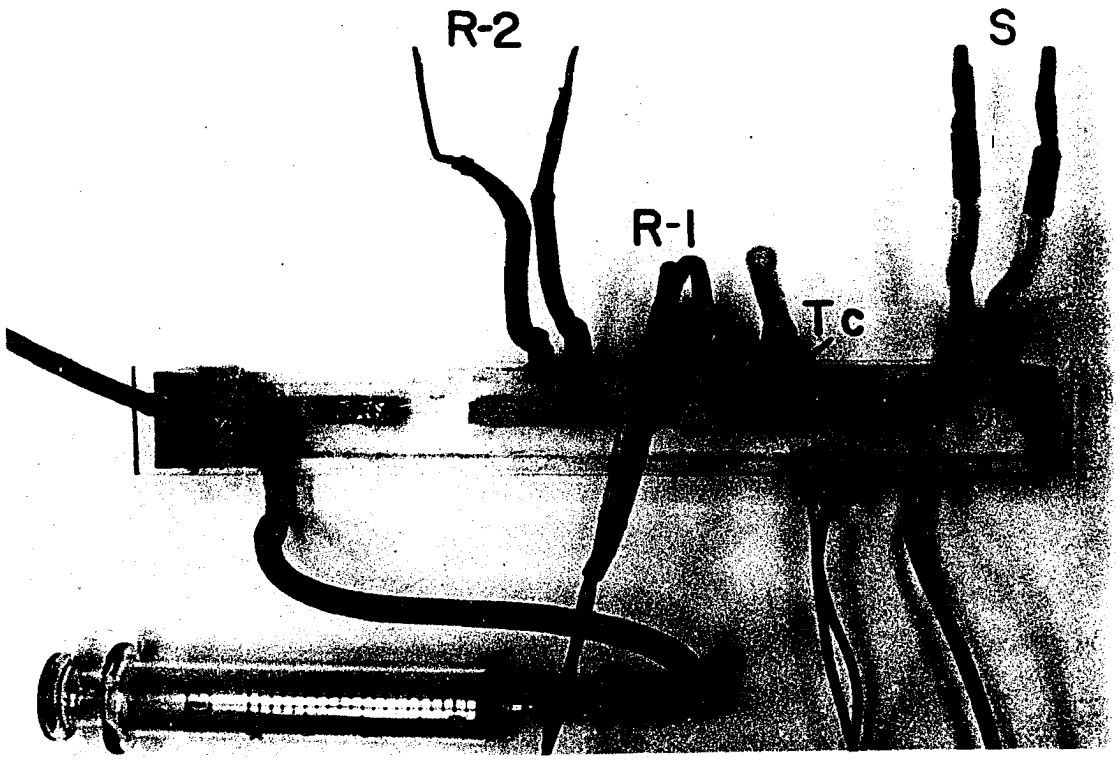

Fig. 1. Immersible nerve chamber used in early studies, including comparison of Alaska and Louisiana muskrats. R1 and R2: recording electrodes; S: stimulating electrodes; Tc: thermocouple.

the temperature of a water bath in which the chamber was immersed. The joint between the base and lid of the chamber was made watertight with chemically inert vacuum wax. Temperatures below 0°C were obtained with a sodium chloride-ice mixture, but control of chamber temperature and cooling rate by this method was somewhat crude. The chamber air temperature was measured with a single iron-constantan thermocouple placed near the center of the nerve.

A second chamber was constructed for use in later studies of muskrats and all other animals. It was machined from a solid aluminum block and had an aluminum cover with double plexiglas viewport (Figure 2). Both the cover and main chamber block were drilled out so that coolant could be circulated through each to change the chamber temperature. Heat exchange between coolant and block proved to be efficient enough so that a chamber air temperature of -5°C could be maintained with a coolant temperature (in the reservoir of the cooling apparatus[1]) of -5.5°C.

The interior of the metal nerve chamber had a plexiglas partition running lengthwise dividing it into two separate chambers. Only one chamber was used, and it

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1. Lauda Ultrakryostat, Lauda Instruments, Inc., Box 119, Westbury, New York, 11590.

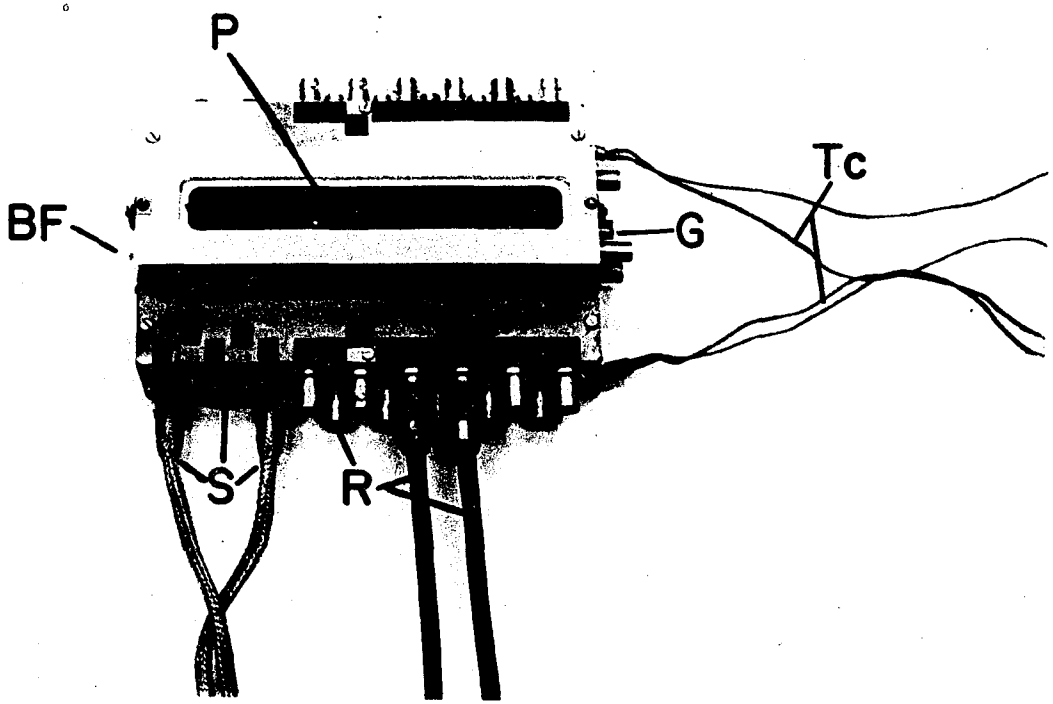

Fig. 2. Metal nerve chamber with internal circulation for temperature control. R: recording electrodes; S: stimulating electrodes; Tc: thermocouples. G: gas inlet; BF: inlet for Locke's solution or saline; P: viewport.

was finally lined with a special room temperature vulcanizing silicone rubber[1] to insulate and protect the nerve and its underlying electrodes from the chamber walls. A small plastic inlet tube at one end of the chamber allowed Locke's or other solutions to be introduced or removed from the chamber, thus allowing the nerves to be periodically rinsed. A small tube at the other end was used to introduce a mixture of 95 percent oxygen/5 percent carbon dioxide. The gas mixture was saturated with water vapor by bubbling it through two water-filled flasks. Each chamber had two iron-constantan thermocouples permanently placed so as to be adjacent to the nerve. Temperatures were recorded in analog form on a Leeds and Northrup Speedomax H[2], or in digital form by a Datex recording system[3]. Thermocouples were calibrated to an accuracy of $\pm$ 0.2°C.

Both the plexiglas and metal nerve chamber were provided with platinum wire electrodes for stimulation and recording. Two sets of recording electrodes in the former

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1. General Electric RTV silicone rubber potting compound, General Electric, Silicone Products Dept., Waterford, New York.

2. Leeds and Northrup Co., 1311 Republican St., Seattle 9, Washington.

3. Datex Corporation, 1307 South Myrtle Avenue, Monrovia, California.

chamber provided recording distances (from the nearest stimulating electrode) of three and seven centimeters. Five stimulating electrodes were present at one end of the metal chamber, allowing a choice of stimulus inter-electrode distance. A separation of one centimeter was used for all experiments. The metal chamber contained 11 recording electrodes spaced at one centimeter intervals, except for a one-half centimeter interval between electrodes three and four, and four and five. A total recording distance of ten centimeters was thus available, if needed. All electrodes were bent in a V shape near the center to provide a trough for the nerve and to allow better contact between nerve and electrodes. Connections between the electrodes and oscilloscope leads were permanent in the plexiglas chamber, while the metal chamber was provided with Amphenol BNC coaxial connectors.

Figure 3 shows the complete experimental system. Nerves were laid on the chamber electrodes and stretched to their in vivo length. The threads attached to each end of the nerve were placed in hooks projecting out from each end of the chamber. The proximal, or larger, end of the nerve was placed on the stimulating electrodes. Stimuli were provided by a Grass Model S-4C stimulator[1]

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1. Grass Instrument Co., 101 Old Colony Ave., Quincy, Mass.

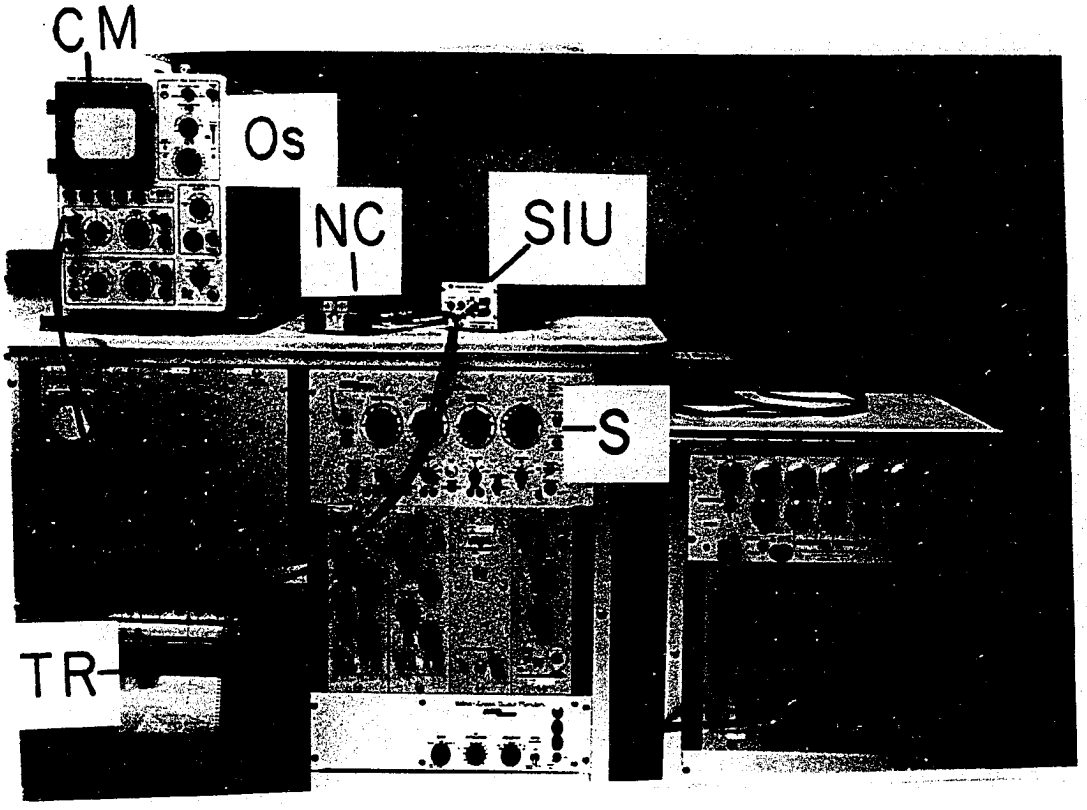

Fig. 3. Instrumentation used in nerve recordings. Os: oscilloscope; S: stimulator; SIU: stimulus isolation unit; NC: nerve chamber; TR: temperature recorder; CM: camera mount for oscilloscope camera.

through a Grass SIU isolation unit. Nerve action potentials were led to a Tektronix Model 502 or 502A[1] dual beam oscilloscope with differential amplification. Internal triggering of the oscilloscope sweep was initiated by the rising portion of the stimulus artifact. No pre-amplification of the nerve potentials was necessary. Photographs of the oscilloscope trace were made with a Tektronix oscilloscope camera[2] with Polaroid back. High contrast Polaroid roll film[3] was used which gave a two tone transparency.

Determination of Conduction Velocity

For the purpose of determining conduction velocity with the greatest degree of accuracy, it is often advisable to record from two sets of recording electrodes at different distances from the stimulating cathode. This technique allows the determination of an "absolute" conduction velocity, i.e., one does not have to take into account the length of time the stimulating current has flowed before an action potential is initiated, or the

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1. Tektronic, Inc., 236, S.W. 153rd St., Seattle, Wash., 98166.
2. Model C12, see note 1.
3. Polaline Type 146-L.

error introduced by the fact that initiation of action potentials in myelinated nerves occurs at nodes of Ranvier, which are usually spaced at intervals of about 0.5 to 2 millimeters (Ruch and Fulton, 1960). In practice, the use of two or more sets of recording electrodes does not circumvent the problem of the change in composition of whole nerve trunks due to branching.

Some of the earlier experiments utilized the recording circuit shown in Figure 4, in which electrodes at two recording positions (R1 and R2) were led into the separate oscilloscope amplifiers. Since monophasic action potentials were desired, a common distal recording lead (R3) was used under which the nerve had been crushed. It was soon discovered that the action potential recorded from the proximal electrode (R1) with the above circuit was shifted slightly, but significantly, on the oscilloscope sweep. The shift was always to the left, resulting in an apparent decrease in the length of time taken for the action potential to move from the stimulus point to R1. This apparent decrease in conduction time caused conduction velocity to be calculated as higher than it really was. The only reason apparent for the action potential shift was a feedback phenomenon between the two amplifiers, but professional electronics consultation

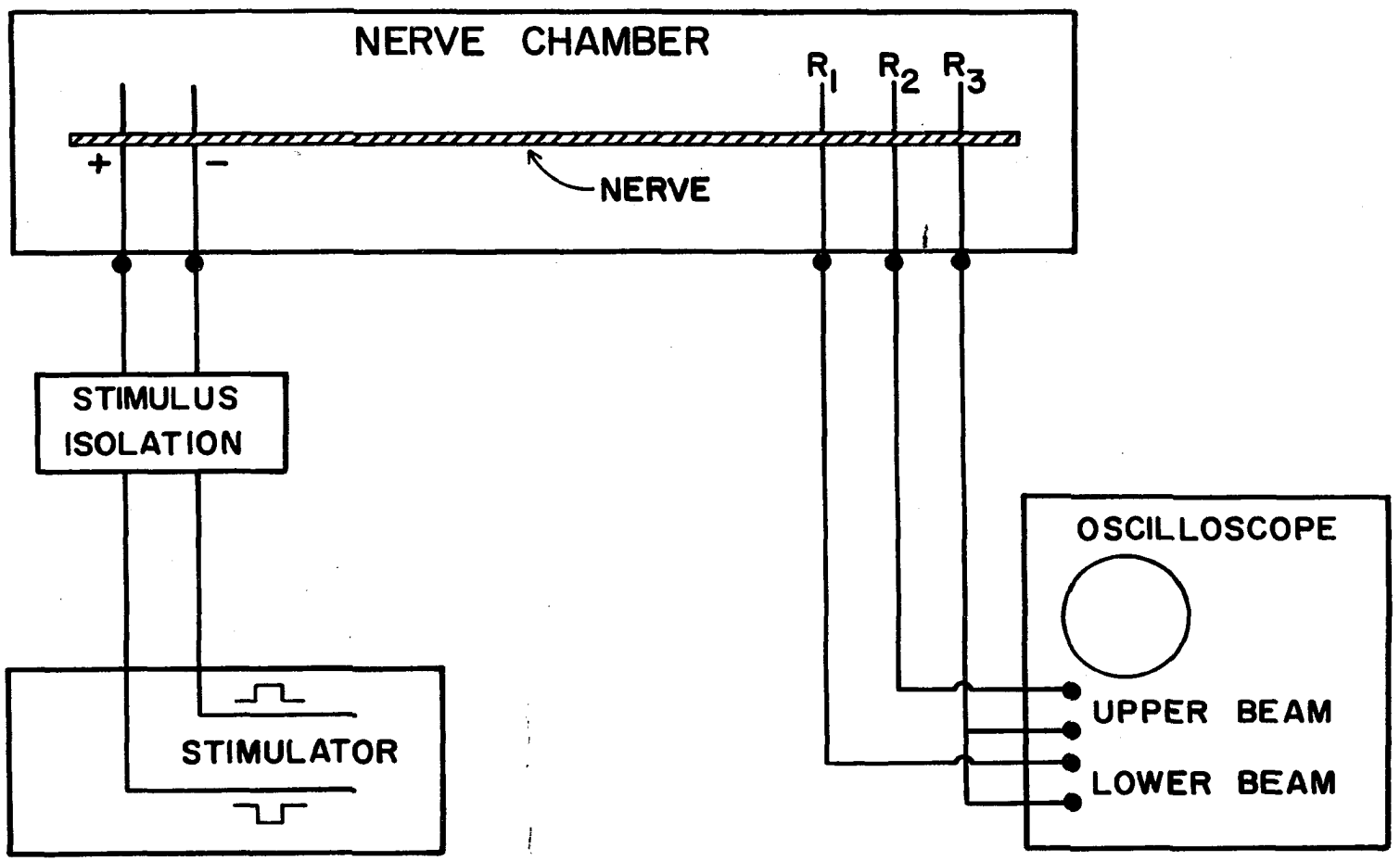

Fig. 4. Circuit used for dual beam presentation of monophasic action potentials.

failed to produce a simple solution to the problem, so this recording method was abandoned.

The larger part of the nerve recordings were accomplished with the use of three electrodes, distal electrode over killed nerve, as mentioned above. However, dual beam presentation of the action potentials was not used, and two separate recordings from the two electrode positions were made consecutively on one beam of the oscilloscope. With this method of recording, absolute conduction velocities could be calculated by determining the difference in time taken by the initial rise of the action potential to reach the two recording electrodes (in milliseconds) and dividing this value into the distance between the electrodes (in millimeters). Conduction velocity thus came out as millimeters per millisecond (mm/msec), or the equivalent, more commonly used expression, meters per second (M/sec).

Conduction velocities were also calculated using the stimulus artifact method, where conduction time between the onset of the stimulus artifact and onset of the action potential was determined from the oscilloscope trace, and this value divided into the distance from stimulating cathodetc recording electrode. These conduction velocity calculations were made for both proximal (electrode

nearest stimulating cathode) and distal (electrode furthest from stimulating cathode) recording electrodes.

Average conduction velocities calculated for each animal from the three methods fell, with a few exceptions, within a range of ten percent. A number were within a range of five percent. The rather close correspondence of the three conduction velocity measurements was somewhat surprising in view of the unknown excitation time for each nerve. If the time required to initiate an action potential occupies a significant portion of the conduction time, the true conduction velocity (or "absolute" conduction velocity) will be higher than the conduction velocity determined from the stimulus artifact method. Following a similar line of reasoning, conduction velocities determined from action potential recordings made close to the stimulus electrodes will be lower and will contain a greater error than velocities determined from more distant recordings. Conduction velocity calculation for some nerves followed this pattern, i.e., proximal electrode recordings gave lower conduction velocites than distal, while "absolute" velocities were still higher. Such results were not consistent for even one animal, however, and many nerves showed completely opposite results.

The most likely explanation for the inconsistent conduction velocity calculations appeared to lie in the small margin of error allowable in making measurements from the oscilloscope trace, particularly in deciding the exact point at which an action potential began. Since conduction velocities determined by one method were not consistently different from values obtained by another and since measurement errors in "absolute" conduction velocity calculations had a greater effect on final results (due to the short conduction distance available, usually one to two centimeters), most of the conduction velocity results are based on stimulus artifact to distal recording electrode measurements.

Excitability Measurements

Nerve excitability was determined at four stimulus durations: 5.8, 1.2, .17, and .03 milliseconds. These were calibrated values representing 5, 1, .1, and .01 milliseconds on the stimulator controls. Determinations made at 5.8 milliseconds were sometimes difficult due to the stimulus artifact overlapping the action potential. At lower temperatures, usually 5°C or below, excitability of many nerves had decreased to the point where a very high stimulus excitation voltage was required at the .01 millisecond stimulus duration. If the voltage requirement

exceeded 25 volts, the excitability for the .01 millisecond stimulus duration was not determined. The excitability threshold was taken as the least stimulus voltage at which an action potential was just visible with an oscilloscope amplification of one millivolt per centimeter. The threshold measured was thus the threshold of the most excitable (and most repidly conducting) fibers.

Due to the fact that impedance can be a significant error factor in excitability measurements made with a stimulator not having constant current stimulus characteristics, tissue-stimulus electrode impedance was determined for several preparations. The method suggested by the Grass Instrument Company (1963) for use with the S-4C stimulator was found to be simple and reliable. It consists of interposing a small resistance (10 ohms or less) across the oscilloscope input and measuring the voltage drop while the nerve is being stimulated. Since the voltage output of the stimulator (without any load) is known and the voltage drop across the known resistance can be measured, the unknown resistance can be easily calculated.

Tissue electrode resistances were invariably higher than 2,500 ohms; and since the output resistance of the Grass stimulator is 250 ohms or less, the stimulus output voltages were considered to be reasonably accurate

Refractory Period

Both absolute and relative refractory periods were determined. The absolute refractory period was necessarily determined for fast fibers, while the relative refractory period applied to the average fiber population. All refractory periods were measured with paired stimuli of 0.2 milliseconds duration, a voltage of ten times threshold, and a frequency of two per second or less. Relative refractory period values could not be determined with as much accuracy as absolute refractory periods. In some nerves, especially at lower temperatures, the second stimulus of a test pair would not produce an action potential as large as the one produced by the first stimulus even though the two stimuli were separated by as much as one second. In such nerves, the relative refractory period was taken as the time at which the action potential no longer showed a noticeable increase in size with a reasonable increase in stimulus interval. The absolute refractory period was used as an index of maximum impulse frequency possible in the fast nerve fibers.

Action Potential Characteristics

The amplitude of compound action potentials is commonly measured in nerve studies with the idea that it will provide a useful measure of functional capability.

Although very gross changes in potential height have some meaning, the interpretation of amplitude changes is complicated by a number of factors and has undoubtedly been subject to much misinterpretation. Ritchie and Straub (1956), by recording action potential amplitude in nonmyelinated fibers at various distances and plotting the amplitude ratio at a given distance for two different temperatures, concluded that little or no change in absolute spike height occurred between 37°C and 25°C even though the recorded height was more than doubled. Their conclusion was based on the idea that at zero and infinite conduction distances the spike amplitude will be determined by a single fiber or a group of synchronously discharging fibers, and the amplitude ratios at two temperatures will be 1.0. Following a similar line of reasoning, it is apparent that a plot of action potential amplitudes made at two different recording distances for each of two temperatures will tend to intersect at greater recording distances, i.e., as infinite recording distance is approached. Since action potentials were recorded from every nerve at two distances at each temperature, plots of similar nature were made for nerves from the various species studied. Several such plots confirmed the findings of Ritchie and Straub, while several did not. It was decided not to

include such plots as part of the dissertation material due to their inconsistency and due to the fact that only two recording distances were used for most nerves.

Typical Recording Procedure for an Excised Nerve

The nerve was mounted in Locke's solution in the nerve chamber at room temperature. Circulating fluid from the Kryostat was allowed to flow through the chamber cooling system at 25°C, and the temperature allowed to stabilize. Locke's solution was then withdrawn and the chamber allowed to stabilize for several more minutes before recording was begun. The 95 percent oxygen/5 percent carbon dioxide mixture was allowed to flow slowly into the chamber during the equilibration period.

General condition of the nerve was checked at several conduction distances and two distances selected for "proximal" and "distal" recording. The action potential height, distance from onset of stimulus artifact to initial action potential rise, and distance from onset of stimulus artifact to action potential peak, were measured at both proximal and distal recording electrodes. These measurements were made using slightly supermaximal (for full development of the initial, usually largest, action potential peak) stimuli of 0.12 milliseconds duration.

Thresholds were then determined for the various stimulus durations starting with 5.8 milliseconds and ending with 0.03 milliseconds. The voltage just necessary to produce a full action potential at each stimulus duration was also determined. Consecutive exposures of proximal and distal action potentials were then made, and the photograph developed. Absolute and relative refractory periods were determined last. Temperature checks were made intermittently during the recording procedure to be sure that the chamber (and nerve) temperature had not varied from the desired temperature by more than $\pm$ 0.2°C.

Locke's solution was then syringed into the chamber to cover the nerve, the oxygen/carbon dioxide mixture turned on, and the temperature of the circulating fluid raised to give a chamber temperature of 35°C. The same procedures were then followed to obtain records at 35°C, 15°C, and usually at 5°C. If the nerve was still conducting an action potential at 5°C, it was cooled immediately following the electrical recordings to a temperature where conduction ceased. An action potential was considered to be absent if not detectable at the highest amplification available, 200 microvolts/centimeter for the 502, and 100 microvolts/centimeter for the 502A oscilloscope.

Cooling rates for chamber air varied between 2° and 0.1°C/minute and were slowest when it could be seen that the action potential was nearly extinct or when a known freezing point for a supercooled nerve was approached. As soon as the action potential disappeared, the chamber temperature was raised to 25°C at a rate of 1° to 3°C/minute. In a few experiments with supercooled nerves, cooling was allowed to proceed below the freezing point after the action potential had disappeared. After the nerve had equilibrated at 25°C, the various electrical characteristics were again determined as a check for deterioration that might have occurred during the experiment. An attempt was also made with some nerves to determine the point during rewarming at which an action potential could again be conducted.

RESULTS

General

The gross physical characteristics of the various animal groups studied are listed in Table 1. Judging from body weight and tail length characteristics it is probable that the Louisiana muskrats were a younger group of animals than the Alaska muskrats. This conclusion is complicated by the fact that the race of muskrats found in Southern Louisiana is somewhat smaller than the more northern races, but it seems justified by the disproportionately low weights of several of the Louisiana animals.

As might be expected the beaver showed the widest range in body size of any group. The two smallest beaver studied, weighing 4.2 and 7.4 kilograms, were animals of the year [using the size criteria of Hakala, (1952)]. They had, therefore, not been exposed to winter cold. All of the remaining beaver were older than one year. Nerves from the smallest beaver did not exhibit any obvious functional differences from nerves of larger animals, and their characteristics are included in the results.

Two coyotes and both porcupines had been in captivity for a considerable length of time (months) before they were utilized for nerve studies.

TABLE 1. General Characteristics of Animals Studied.

Group	No.	Sex ♂	Sex ♀	Body Weight(Kg)	Tail Length(cm)
Alaska Muskrats	11	5	4	.934 (.800 to 1.090)	23.1 (20 to 25)
La. Muskrats	12	2	3	.744 (.575 to 1.001)	20.8 (18 to 23.5)
Alaska Muskrats (outdoor)	13	9	4	.942 (.775 to 1.040)	23.6 (22.5 to 24.5)
Alaska Muskrats (indoor)	5	2	2	.921 (.730 to 1.180)	22.8 (22 to 24.5)
Beaver	12	7	4	13.16 (4.20 to 23.90)	31.7 (18 to 39)
Red-Squirrel	9	5	4	.211 (.188 to .232)	12.0 (9.2 to 13)
Red Fox	13	9	4	4.79 (3.00 to 5.80)	40.2 (35 to 46)
Coyote	3	2	1	12.75 (10.00 to 15.70)	31.8 (28 to 39)
Marten	2	2		.990 (.815 to 1.165)	16.3 (14.7 to 17.9)
Porcupine	2		2	11.70	
Mink	1	1		1.040	
Moose	1		1	Est.300	

The animals in which tail temperatures were measured during cold exposure fell into the pattern previously suggested (Table 2). The heavily furred coyote tail showed a subcutaneous temperature near the tip that was about 20°C higher than the tail tip of a red squirrel after similar exposure. The marten tail showed intermediate temperature characteristics. Several attempts have been made to measure tail temperatures in beaver in various thermal environments. Steen and Steen (1965) recently published a short communication on the thermoregulatory importance of the beaver's tail. Only one very young (2.8 kilogram) animal was studied, but the results showed that tail temperature could drop to 8°C with the tail immersed in 6°C water. In warm air and without cold water available, the tail temperature rose to 38°C.

Temperatures of 4° to 6°C have been measured in the tails of larger, free swimming beaver in a near-freezing bath (Miller, unpublished observations).

Representative monophasic action potentials for the different types of nerve studied in each species are shown in Figure 5a, 5b, and 5c. Most of the caudal nerve compound action potentials show only one smooth, distinct peak, indicating a single major fiber group. A fairly distinct later peak, usually much smaller, could be seen

TABLE 2. Subcutaneous Temperatures of Several Animals (°C) Following Cold Exposure.*

Species	Specimen No.	Air Temp.	Exposure Time(min)	Deep Body Temp.	Tail Temps.			Thigh Temps.		
					Base	Mid	Tip	Upper	Mid	Lower
Marten	64-2	-15°C	60	37.5	28.0		12.5	34.0		
Coyote	64-3	-25°C	90	39.0	33.5	28.5	28.0	38.5		33.5
Red Squirrel	64-5	-30°C	50	40.5	13.0		7.0	40.5		20.5
Red Squirrel	65-1	-12°C	45	39.5	17.5		13.0		38.0	

*All temperatures measured within 1 minute after death.

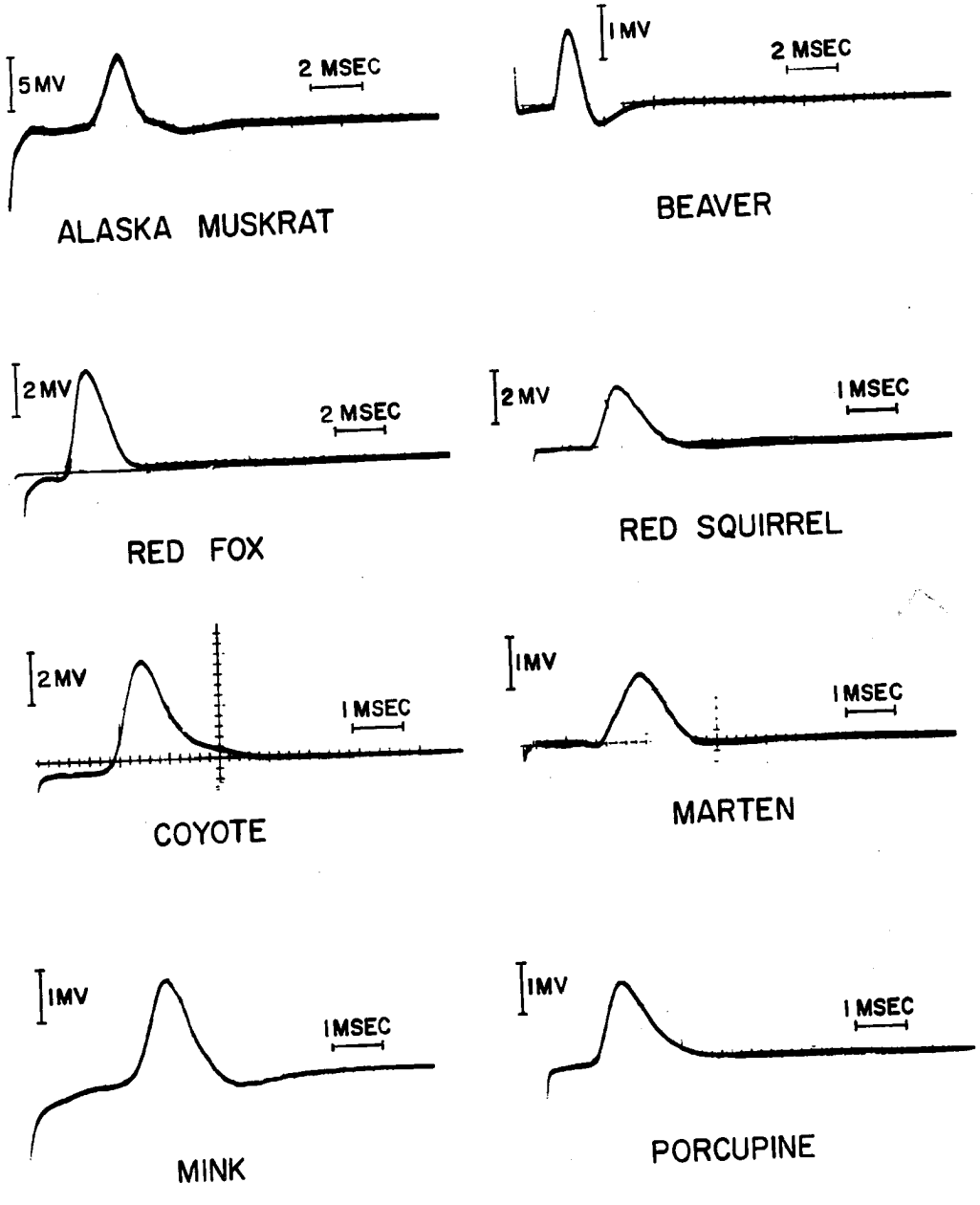

Fig. 5a. Representative action potentials, caudal nerve, 25°C.

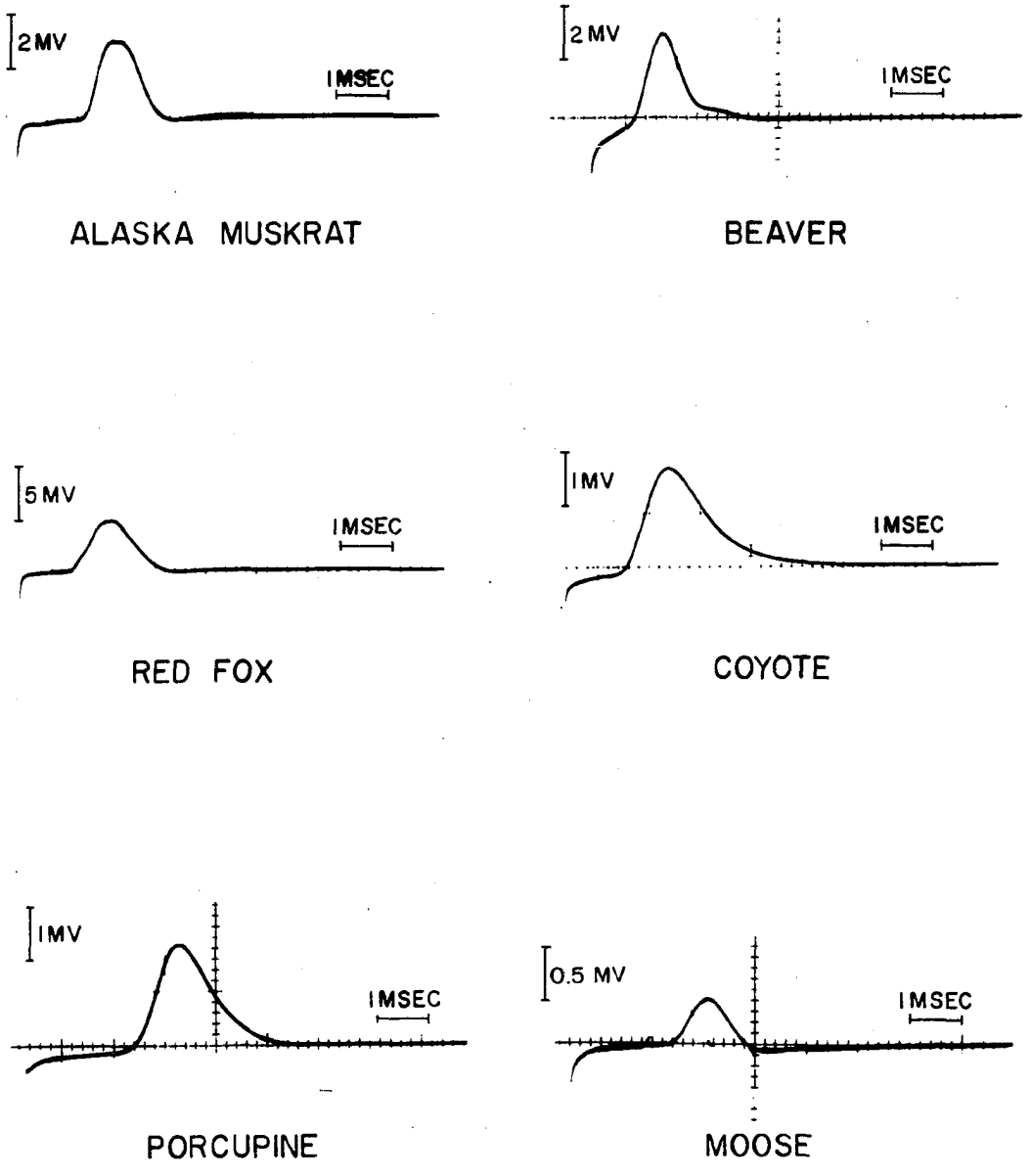

Fig. 5b. Representative action potentials, tibial nerve, 25°C.

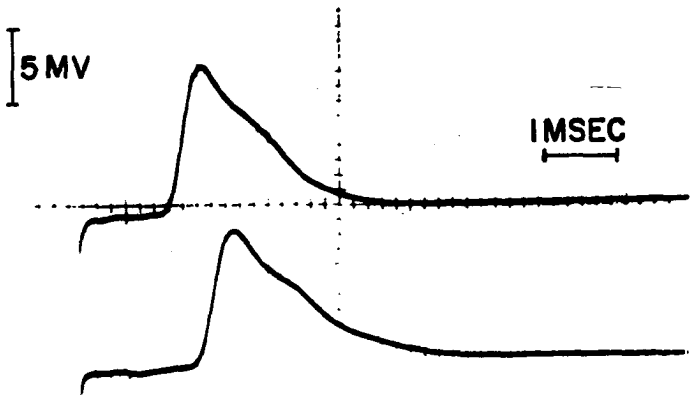

Fig. 5c. Typical beaver phrenic nerve action potential, 25°C.

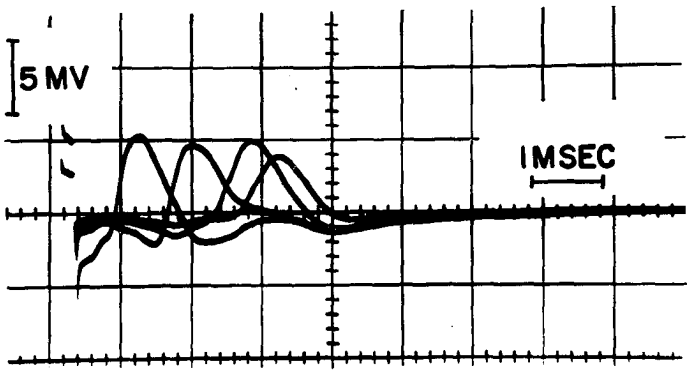

Fig. 6. Action potentials (muskrat caudal nerve) recorded at distances of 2, 4, 6, and 7 centimeters from stimulating cathode, 25°C.

in several tibial and phrenic nerves. The main action potential peaks of the tibial nerves were not infrequently rounded, indicating the presence of two or more fiber groups or a wider fiber distribution.

In several instances caudal nerve action potentials showed partially diphasic or even triphasic waveforms. Diphasic potentials can be attributed to incomplete killing of the nerve under the reference recording electrode and in some cases to poor grounding of the preparation. Triphasic waveforms are commonly encountered in volume conductors and their presence, usually slight, was probably due to Locke's solution adhering to the nerve. Diphasic and triphasic waveform alterations are not believed to have introduced serious error into action potential amplitude determinations and could not have affected other measurements.

Stimulus artifacts occasionally caused some minor problems, particularly where recording distances were short (under two centimeters). Some were apparently due to ground loops caused by an imperfectly insulated nerve chamber; others may be attributed to lack of a good nerve ground.

In most nerves action potential amplitude was smaller at greater recording distances, as would be

expected due to time dissociation of the action potential components and progressive dropping out of individual fibers. Exceptions to the general condition did occur, as may be seen in muskrat 65-2; Table 3 and Figure 6. At 15°and 5°C action potential height was greater at a recording distance of six centimeters than at four centimeters. Such anomalies were probably due to variations in electrode contact or to localized nerve injury.

The effect of alterations in recording distance on conduction velocity determinations was carefully checked in two animals (Table 4). Results indicate that no consistent velocity changes occur with changes in recording distance as long as the distance is more than three centimeters. The lower conduction velocity values in muskrat 65-3 at two centimeters recording distance are attributed to stimulus artifact influence on the early portion of the action potential, making the determination of the exact point of initial action potential rise uncertain.

Cooling had no detrimental effect on the viability of most nerves. As shown in Table 5, noticeable decreases in conduction velocity after cooling followed by rewarming to 25°C occurred only in the red squirrel and mink. Some decrease is to be expected as a consequence of the slow

TABLE 3. Action Potential Height (mv) at Various Recording Distances and Temperatures, Caudal Nerve.

Animal	Recording Distance (cm)	Temperature (°C)				
		35°	25°	15°	5°	-5°
Muskrat 65-3	2	5.0	5.5	6.0	4.5	
"	4	4.6	5.0	5.3	3.9	
"	6	4.5	5.0	6.0	4.4	
"	7	3.5	4.0	4.8	3.8	1.5
Coyote 64-2	3		6.0			
"	4		5.0			
"	5		4.6			
"	6		3.7			
"	7		2.8			

TABLE 4. Conduction Velocities (M/sec) at Various Electrode Distances, Caudal Nerve.

Animal	Recording Distance (cm)	Nerve Temperature (°C)			
		35°	25°	15°	5°
Coyote 64-2	3.0		40.0		
"	4.0		44.4		
"	5.0		41.7		
"	6.0		40.0		
"	7.0		40.0		
"	8.5		40.0		
Muskrat 65-3	2.0	33.3	25.0	16.7	8.9
"	4.0	38.1	26.7	17.4	9.4
"	6.0	38.7	26.7	17.6	9.3
"	7.0	38.9	26.4	17.5	9.0

TABLE 5a. All Groups, Pre- and Post-Cooling Comparisons, Caudal Nerves. 25°C.

Group	No.	Conduction Velocity (M/sec)		No.	Absolute Refractory Period (msec)	
		Pre-Cooling	Post-Cooling		Pre-Cooling	Post-Cooling
Alaska Muskrats	9	26.3	25.8	10	1.0	.9
La. Muskrats	12	30.7	32.0	12	1.0	1.0
Alaska Muskrats (outdoor)	9	25.5	25.9	10	.67	.69
Beaver	6	31.9	30.1	7	.71	.70
Red Squirrel	6	26.0	21.9	9	.65	.90
Red Fox	13	37.2	34.9	13	.82	.92
Coyote	3	37.6	38.0	3	.67	.66
Marten	1	30.0	30.0	1	1.4	.65
Porcupine	2	27.4	27.1	2	1.1	1.0
Mink	1	29.7	25.0	1	.60	.65
Moose						

All values are mean.

TABLE 5b. All Groups, Pre- and Post-Cooling Comparisons, Tibial Nerves, 25°C.

Group	No.	Conduction Velocity (M/sec)		No.	Absolute Refractory Period (msec)	
		Pre-Cooling	Post-Cooling		Pre-Cooling	Post-Cooling
Alaska Muskrats (outdoor)	6	26.9	23.4	8	.73	.72
Beaver	7	31.6	29.3	6	.75	.70
Red Fox	9	42.2	41.2	9	.8	.7
Coyote	2	43.7	43.2	3	.68	1.23
Moose	1	16.2	16.2	1	2.1	2.0

All values are mean.

deterioration with time that occurs in all excised nerves. The red squirrel and fox show sizeable increases in absolute refractory period, which may be taken as another indication of deterioration following cooling and rewarming. The consistent changes in function seen in the red squirrel caudal nerve are best attributed to the fact that their small size and close attachment to intervertebral cartilage makes them more prone to minor damage during removal. Such damage would be expected to speed up deteriorative changes due either to time or low temperature.

Cooling and rewarming of tibial nerves was associated with an increase in absolute refractory period only in the coyote. It is difficult to find any correlation between loss of function in the coyote tibial nerve and morphological or physiological characteristics unique to that animal.

A typical example of a nerve that was little affected by cooling and rewarming, as evidenced by the small change in action potential characteristics (measured at 25°C) before and after cooling to -2°C, is seen in Figure 7A and 7B.

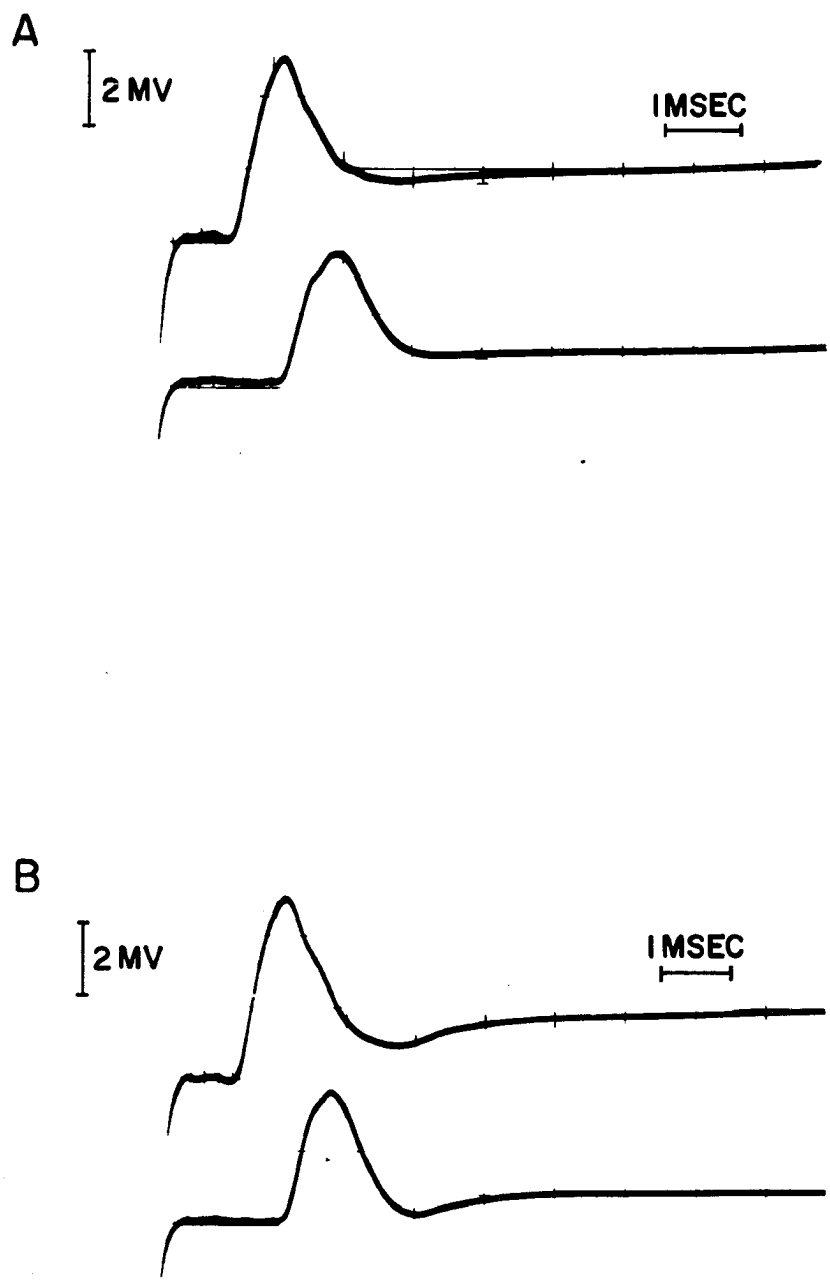

Fig. 7. Tibial nerve action potentials recorded at 25°C, before (A) and after (B) cooling to -2°C.

Temperature-Related Nerve Function in Some Selected Alaskan Mammals

One of the best indicators of temperature related differences in different types of nerves, i.e., in caudal, tibial, and phrenic, as well as of differences between the same type of nerve in different species is the temperature at which nerve conduction ceased. These values are listed in Table 6. In a number of tests nerve conduction was found at temperatures well below the freezing point of animal tissue, which is approximately -0.6° to -0.8°C. This unexpected finding held true for the majority of caudal nerves studied. Some tibial nerves also conducted at temperatures below freezing, but except for the single moose tibial nerve, conduction was not present at the low (-4° to -7°C) temperatures at which it was found in caudal nerves. It should be noted that a number of the action potential extinction temperatures for caudal nerves actually represent the point at which freezing occurred (accompanied by spontaneous rewarming of the nerve). Well developed action potentials were present in some instances just prior to freezing, indicating capability of the nerves to conduct at lower temperatures if freezing could have been prevented.

TABLE 6. Lowest Temperature at Which Action Potential Was Present (°C).

Group	No.	Caudal	No.	Tibial
Alaska Muskrats (outdoor)	13	-4.4[a] (-3.4 to -5.4)	9	1.7 (8.2 to -1.3)
Alaska Muskrats (indoor)	5	-4.6[a] (1.2 to -6.7)		
Beaver	9	-5.0[b] (-3.8 to -5.6)	7	-0.7 (3.8 to -3.8)
Red Squirrel	8	-3.8[c] (3.1 to -6.0)		
Red Fox	13	3.1 (8.0 to -1.3)	9	3.6 (8.5 to 0.3)
Coyote	3	1.0 (4.2 to -1.5)	3	2.7 (3.0 to 2.5)
Marten	2	-4.7 (-4.3 to -5.0)		
Porcupine	2	2.5 (4.5 to0.5)	1	4.3
Mink	1	-5.1		
Moose			1	-5.0[d]

Group	No.	Phrenic
Beaver	6	4.5 (1.0 to 6.8)

All values listed are mean, with range in parenthesis.
a) Nerves still conducting (action potential present) in 3 cases.
b) Nerves still conducting (action potential present) in 5 cases.
c) Nerves still conduction (action potential present) in 5 cases.
d) Nerve still conducting.

The manner in which action potentials disappeared was variable. In some cases the action potential amplitude at a temperature only several degrees above the extinction point was almost equal to the maximal amplitude seen at 15° to 25°C. In other tests the amplitude would decrease evenly from a temperature near 15°C to the extinction point. Some action potentials showed a rapid initial drop at a temperature of 10°C above the extinction temperature but retained a small action potential until final extinction. A thorough study of the effect of cooling rate on action potential extinction was not made, but the importance of cooling rate in temperature studies is well known. Some effects of altered cooling rate were observed even within the rather small range utilized.

No cooling rates used in the studies would be considered rapid by cryobiological standards. The highest cooling rates approached 2°C/minute, and such rates resulted in the lowest action potential extinction temperatures obtained. Several nerves ceased conducting when held at constant or nearly constant low temperature. One muskrat nerve failed after remaining at -2°C for about ten minutes.

After action potential extinction, during artificial rewarming the action potential did not usually

reappear at eitherthe melting point or at the temperature at which it first disappeared, but usually reappeared at a considerably higher temperature. This phenomenon will be mentioned further in the section on Alaska-Louisiana muskrat comparisons.

Figure 8 is a multiple exposure of action potentials (muskrat caudal nerve) recorded at 10°C intervals between 35° and 5°C showing the gross changes in conduction rate and waveform that occurred over the usual temperature test range. These changes will be considered in detail in the following paragraphs.

Conduction velocities of various nerves at different temperatures reflect both the fiber makeup and extinction temperature characteristics. Tables 7, 8, and 9 list caudal, tibial, and phrenic nerve conduction velocities, respectively, for each species at different temperatures. These values for caudal and tibial nerves are plotted for visual comparison in Figures 9, 10, and 11.

In all cases, those nerves which conduct to the lowest temperatures show lower conduction velocity-temperature slopes. This is explained in part by the fact that conduction velocity reaches zero at a lower temperature in such nerves. The much steeper conduction velocity slopes of the red fox and coyote nerves derive not only from

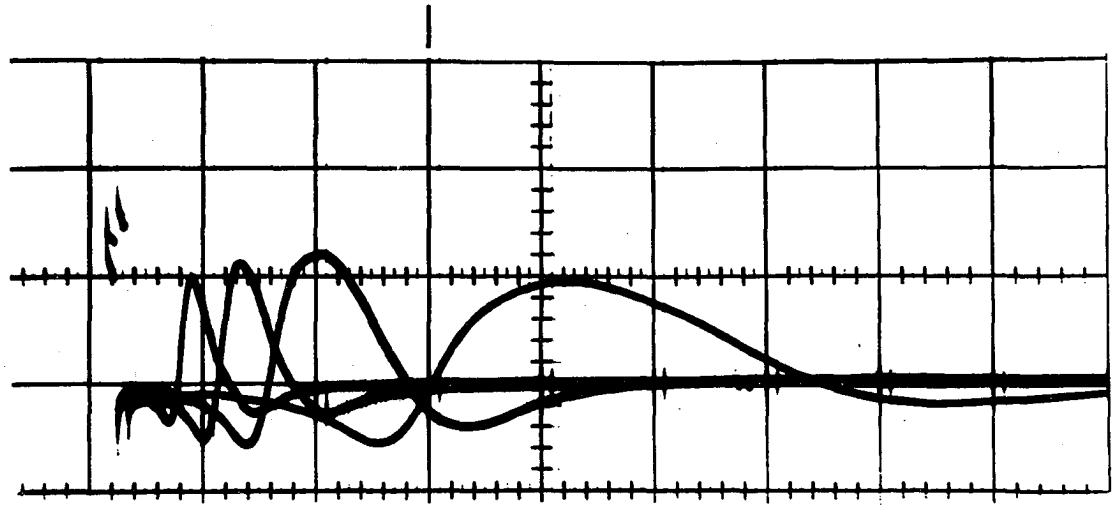

Fig. 8. Muskrat caudal nerve action potentials recorded at temperatures (left to right) of 35°, 25°, 15°, and 5°C. Multiple exposure; sweep, 2 milliseconds/centimeter; amplification, 5 millivolts/centimeter.

TABLE 7. Caudal Nerve Conduction Velocity (M/sec) as Related to Temperature.

Group	No.	Temperature			
		35°	25°	15°	5°
Muskrats (outdoor)	13	34.9±1.0	25.5±0.7	16.6±0.5	8.6±0.3
Muskrats (indoor)	5	35.3±0.8	26.0±0.7	16.4±0.6	7.4±0.4*
Beaver	9	42.9±1.7	31.6±1.3	19.6±0.8	10.0±0.5
Red Squirrel	8	35.8±1.4	26.3±0.8	15.5±0.9	8.0±0.7
Red Fox	13	54.0±1.4	37.2±1.0	20.9±0.6[a]	3.6±0.5[b]
Coyote	3	54.2	37.6	22.8	5.3
Marten	2	44.8	31.6	19.7	8.8
Porcupine	2	39.0	27.4	12.0	1.5
Mink	1	41.5	29.7	20.4	9.6

Values are mean ± standard error.
*Significantly different from outdoor muskrats, $P < .005$.
a) Six animals
b) Four animals

Q_{10} Values for Caudal Nerve Conduction Velocity

Group	Temperature Interval		
	5° to 15°	15° to 25°	25° to 35°
Muskrats (outdoor)	1.98	1.54	1.37
Muskrats (indoor)	2.21	1.58	1.36
Beaver	1.96	1.61	1.36
Red Squirrel	1.94	1.70	1.36
Red Fox	6.36	1.78	1.45

TABLE 8. Tibial Nerve Conduction Velocity (M/sec) as Related to Temperature.

Group	No.	Temperature			
		35°	25°	15°	5°
Muskrats (outdoor)	9	35.8±1.4	26.5±0.9	15.0±0.7	4.4±0.5
Beaver	7	44.6±2.6	31.6±2.4	20.0±1.4	8.1±1.0
Red Fox	9	62.8±2.5	42.1±1.6	22.9±4.7[a]	4.9±2.1[b]
Coyote	3	62.8	44.0	24.8	7.0
Porcupine	1	47.2	28.3	17.7	3.4
Moose	1	39.6	28.3	16.2	7.6

Values are mean ± standard error.
a) Three animals
b) Two animals

Q_{10} Values for Tibial Nerve Conduction Velocity

Group	Temperature Interval		
	5° to 15°	15° to 25°	25° to 35°
Muskrats (outdoor)	3.41	1.77	1.35
Beaver	2.65	1.60	1.44
Red Fox	6.47	1.84	1.49

TABLE 9. Phrenic Nerve Conduction Velocity (M/sec) as Related to Temperature.

Group	No.	Temperature			
		35°	25°	15°	5°
Beaver	6	49.6±1.6	33.4±1.9	18.8±1.2	3.3±0.9

Values are mean ± standard error.

Q_{10} Values for Phrenic Nerve Conduction Velocity

Group	Temperature Interval		
	5° to 15°	15° to 25°	25° to 35°
Beaver	10.6	1.72	1.54

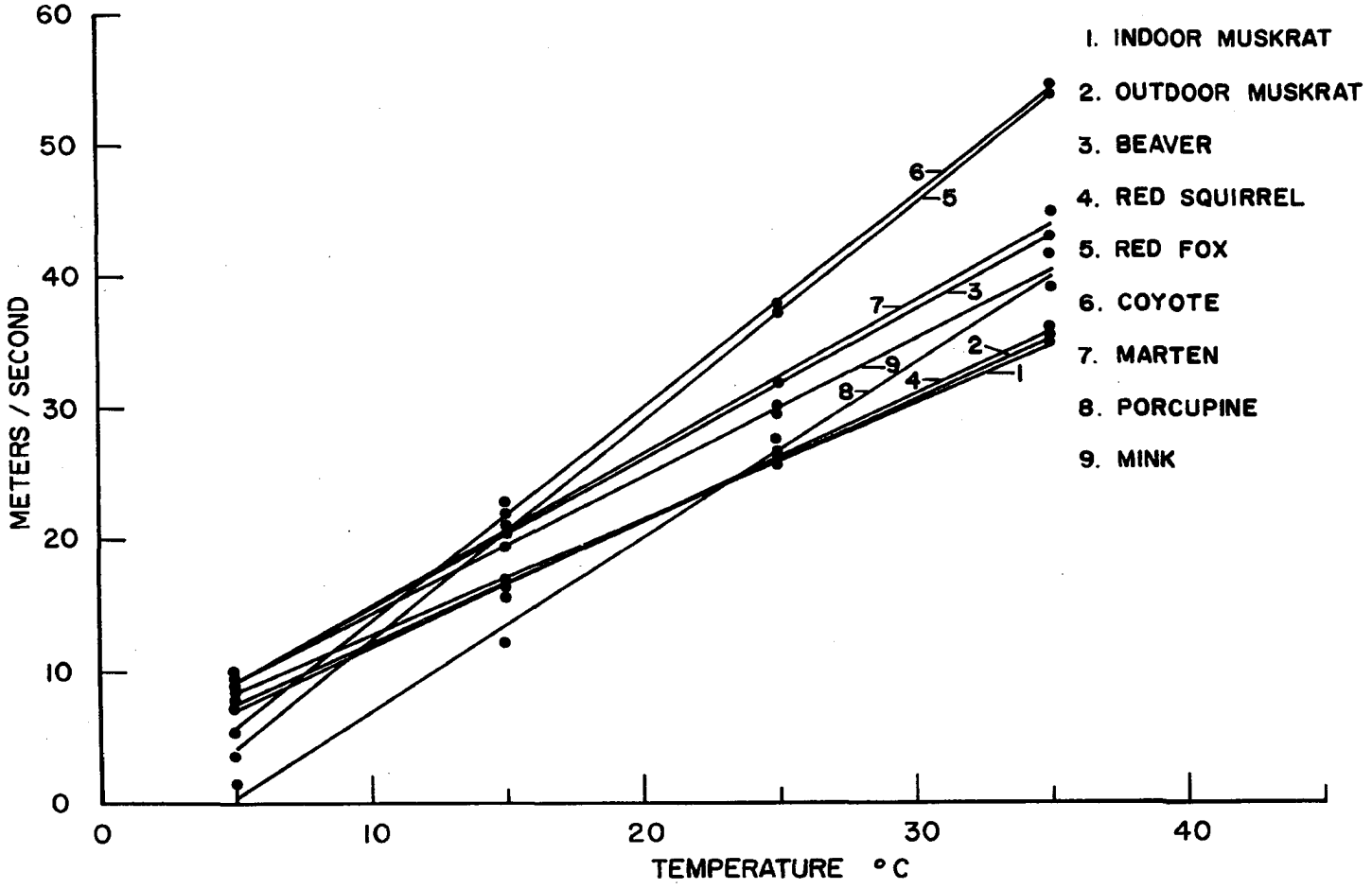

Fig. 9. Caudal nerve conduction velocity as related to temperature, Alaskan mammals.

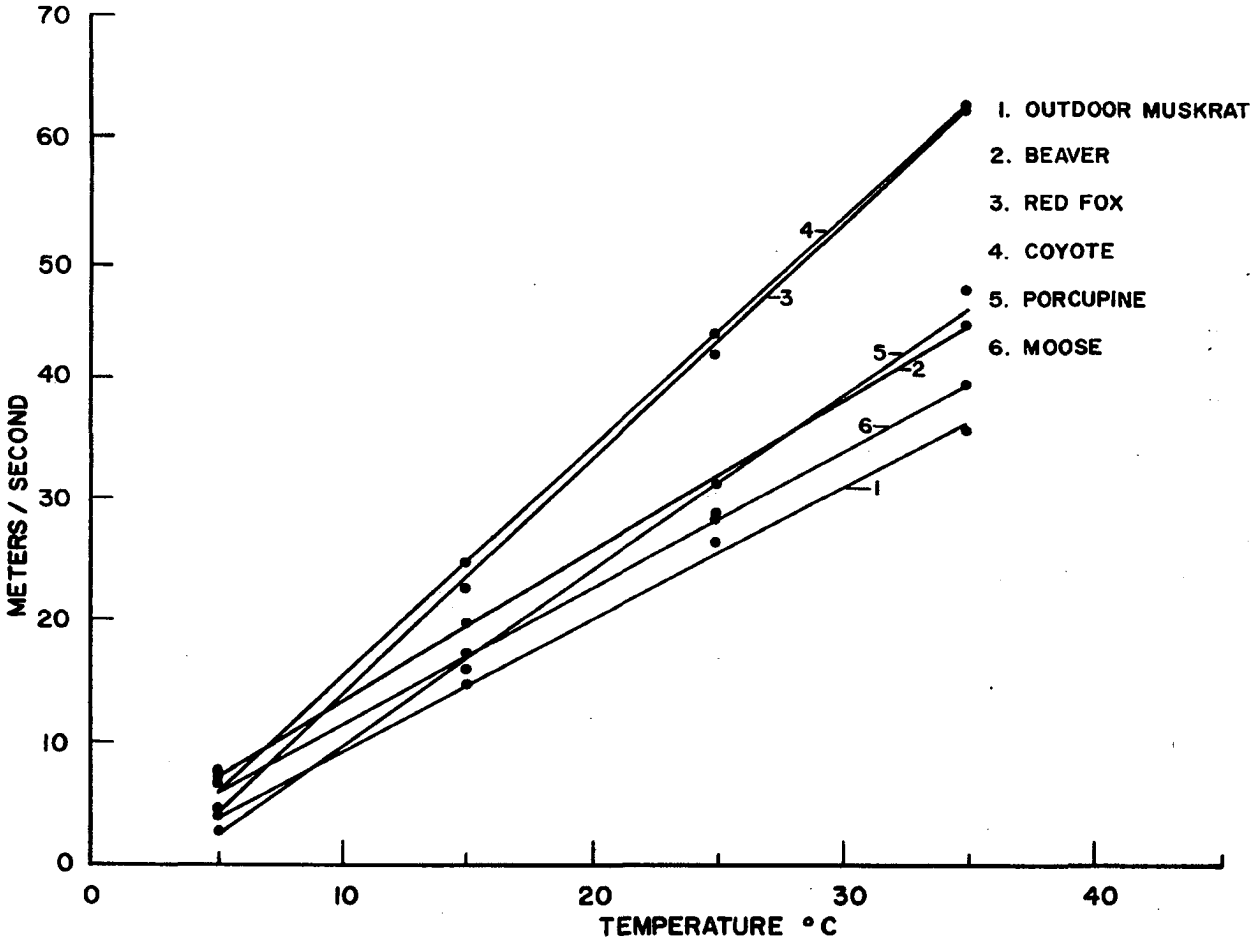

Fig. 10. Tibial nerve conduction velocity as related to temperature, Alaskan mammals.

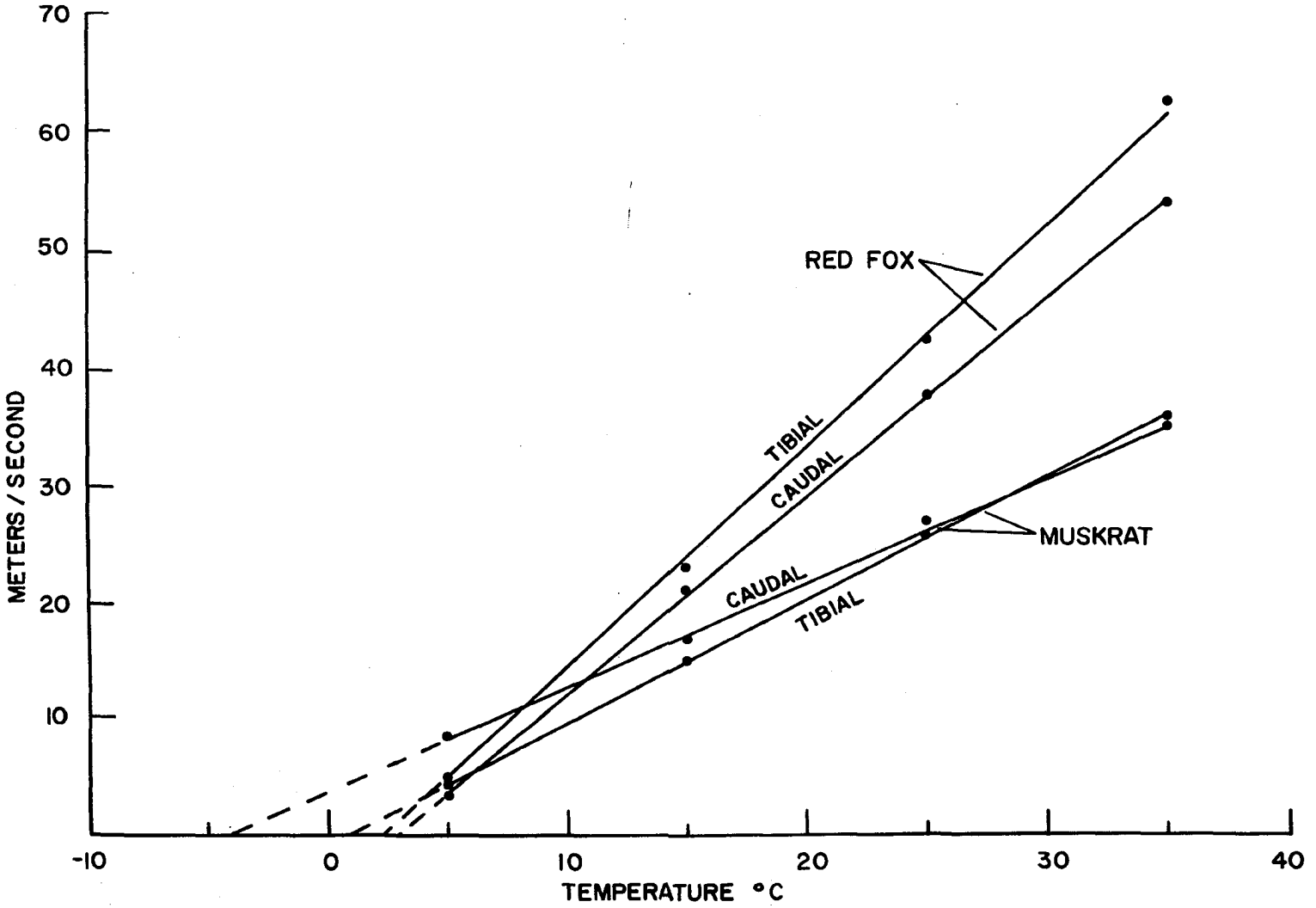

Fig. 11. Caudal and tibial nerve conduction velocities as related to temperature, red fox and muskrat.

higher action potential extinction temperatures but also from the larger overall size of the component fibers. The latter point is evidenced by the fact that both caudal and tibial nerves of the red fox and coyote have conduction velocities at 35°C of over 50 and 60 meters per second, respectively. Extrapolation of the conduction velocity curves to zero yields temperature axis intersection values that correspond closely with actual action potential extinction temperatures.

Differences between tibial and caudal (or in the case of the beaver between tibial, caudal, and phrenic) nerves in the same animal point up the ability of the caudal nerves to function at lower temperatures. This is true to an even greater degree for those animals in which the tails undergo large temperature fluctuations. In all cases the tibial nerves have steeper slopes than do caudal nerves. In the beaver, the phrenic nerve shows a considerably greater slope than the tibial (Figure 12), a feature perhaps related to the constant high temperature of the thoracic cavity.

Tables 7, 8, and 9 also include mean Q_{10} values for the various temperature test intervals between 5° and 35°C. As indicated by the straight line conduction velocity functions, Q_{10} values for all nerves increase

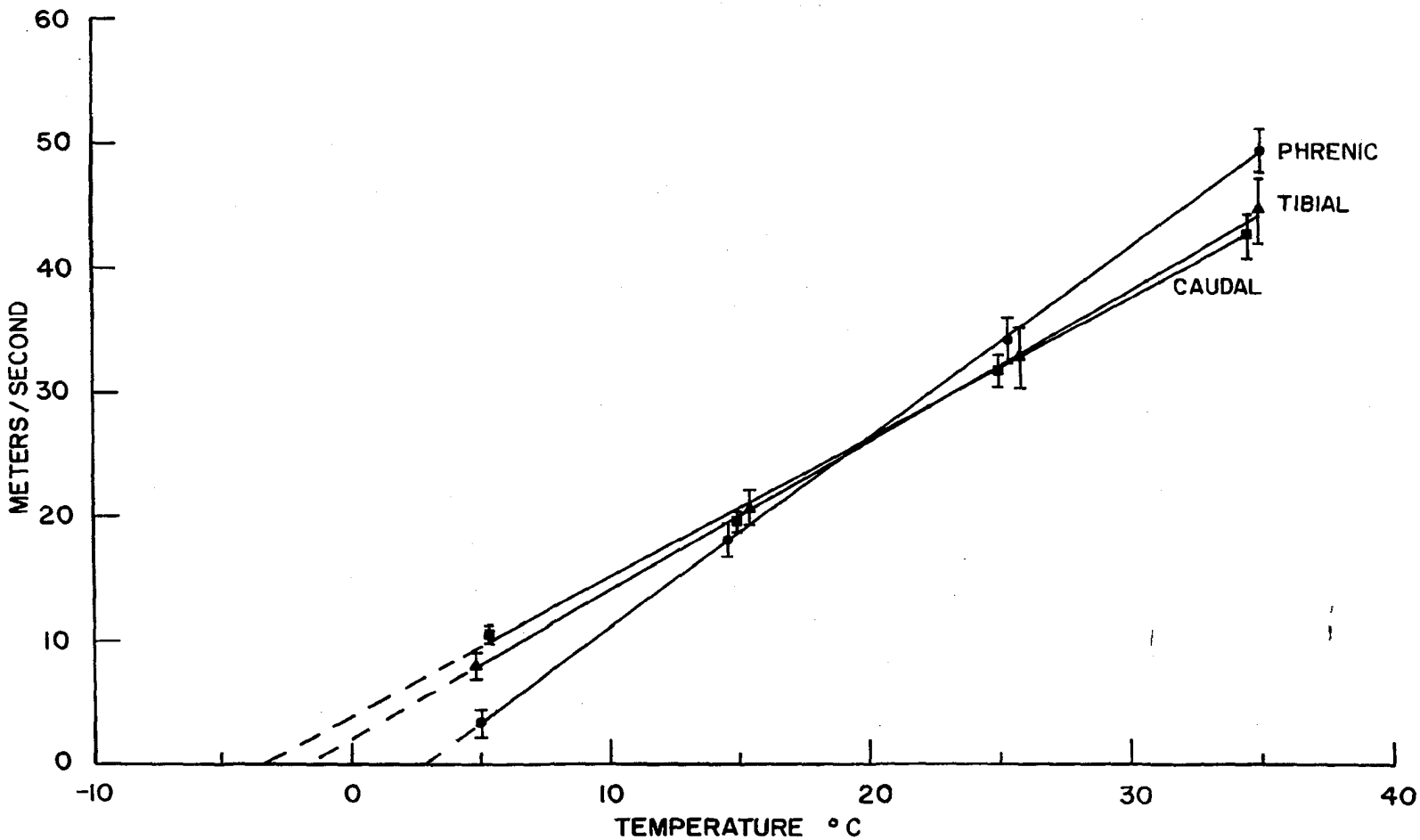

Fig. 12. Caudal, tibial, and phrenic nerve conduction velocities as related to temperature; beaver. Dashed portions of curves are extrapolated to zero conduction velocity.

with decreasing temperatures. The increase is less in those nerves which conduct at the lowest temperatures, i.e., most of the caudal nerves. The fox caudal nerves, which could not function at lower temperatures, showed very high Q_{10} values in the 5° to 15°C temperature interval. Similar values are shown by the fox tibial nerve. The highest Q_{10} values are seen in the phrenic nerve in the 5° to 15°C temperature range.

Excitability tests showed that only relatively minor variations were present in various nerves with respect to temperature relations of this characteristic (Tables 10 through 17). The usual strength-duration characteristics are somewhat altered by lower temperatures (Figures 13 and 14), both by the shifting upward of all values and by a differential effect at longer stimulus durations. As an example of the latter, the threshold voltages for outdoor muskrat caudal nerves are the same at 35°C for stimulus durations of 5.8 and 1.2 milliseconds. They are also the same at 25°C for these stimulus durations. At 15°C, however, and to a greater extent at 5°C, threshold voltages increase markedly between stimulus durations of 5.8 and 1.2 milliseconds. As might be expected, low temperature had a greater effect on stimuli of short duration than those of longer duration. This is

TABLE 10. Caudal Nerve Excitability Threshold (Volts) as Related to Temperature, 5.8 msec Stimulus Duration.

Group	No.	Temperature			
		35°	25°	15°	5°
Muskrats (outdoor)	9	.021±.005	.055±.008	.093±.019	.105±.029
Beaver	7	.042±.006	.047±.006	.102±.020	.311±.041
Red Squirrel	9	.0731±.018	.060±.011	.12±.02	.15±.02
Red Fox	11	.0551±.014	.060±.012	.045±.066	.13[a]±.038
Coyote	3	.028	.059	.080	.162
Marten	2	.042	.034	.11	.10
Porcupine	2	.21	.22	.26	.5
Mink	1	.056	.045	.088	.08

Values are mean or mean ± standard error.
a) Nine animals

Q_{10} Values for Caudal Nerve Excitability

Group	Temperature Interval		
	5° to 15°	15° to 25°	25° to 35°
Muskrats (outdoor)	1.13	1.69	2.61
Beaver	3.05	2.17	1.12
Red Squirrel	1.25	2.00	.82
Red Fox	2.89	.75	1.09
Coyote	2.02	1.36	2.11
Marten	.91	3.24	.81
Porcupine	1.92	1.18	1.05
Mink	.91	1.96	.80

TABLE 11. Caudal Nerve Excitability Threshold (Volts) as Related to Temperature, 1.2 msec Stimulus Duration.

Group	No.	Temperature			
		35°	25°	15°	5°
Muskrats (outdoor)	11	.020±.004	.060±.007	.12±.02	.20±.03
Muskrats (indoor)	5	.020±.004	.038±.006	.08±.01	.13±.03
Beaver	9	.040±.004	.050±.006	.15±.02	.36±.02
Red Squirrel	9	.070±.020	.070±.014	.24±.10	.21±.02
Red Fox	13	.049±.011	.057±.010	.056±.004	.20[a]±.05
Coyote	3	.031	.060	.09	.26
Marten	2	.042	.037	.14	.16
Porcupine	2	.26	.22	.27	.66
Mink	1	.05	.049	.11	.23

Values are mean or mean ± standard error.
a) Eleven animals

Q_{10} Values for Caudal Nerve Excitability

Group	Temperature Interval		
	5° to 15°	15° to 25°	25° to 35°
Muskrats (outdoor)	1.67	2.00	3.00
Muskrats (indoor)	1.62	2.10	1.90
Beaver	2.40	3.00	1.25
Red Squirrel	.88	3.43	1.00
Red Fox	2.48	.98	1.16
Coyote	2.88	1.50	1.94
Marten	1.14	3.78	.88
Porcupine	2.44	1.23	.85
Mink	2.09	2.24	.98

TABLE 12. Caudal Nerve Excitability Threshold (Volts) as Related to Temperature, .17 msec Stimulus Duration.

Group	No.	Temperature			
		35°	25°	15°	5°
Muskrats (outdoor)	11	.087±.018	.22±.03	.38±.04	.59±.05
Muskrats (indoor)	5	.06±.01	.14±.03	.32±.02	.45±.06
Beaver	9	.16±.02	.23±.02	.38±.02	.86±.10
Red Squirrel	9	.17±.04	.21±.03	.38±.02	.63±.03
Red Fox	13	.091±.014	.17±.018	.20±.016	.75[a]±.019
Coyote	3	.10	.18	.29	1.0
Marten	2	.17	.17	.40	.68
Porcupine	2	.39	.43	.61	2.6
Mink	1	.20	.23	.44	.76

Values are mean or mean ± standard error.
a) Eleven animals

Q_{10} Values for Caudal Nerve Excitability

Group	Temperature Interval		
	5° to 15°	15° to 25°	25° to 35°
Muskrats (outdoor)	1.55	1.73	2.53
Muskrats (indoor)	1.41	2.28	2.33
Beaver	2.26	1.65	1.44
Red Squirrel	1.66	1.81	1.24
Red Fox	3.75	1.18	1.87
Coyote	3.45	1.18	1.80
Marten	1.70	2.35	1.00
Porcupine	4.26	1.42	1.10
Mink	1.73	1.91	1.15

TABLE 13. Caudal Nerve Excitability Threshold (Volts) as Related to Temperature, .03 msec Stimulus Duration.

Group	No.	Temperature			
		35°	25°	15°	5°
Muskrats (outdoor)	11	.37±.04	.65±.05	1.7±.29	3.0±0.2
Muskrats (indoor)	5	.26±.03	.50±.07	0.9±.05	2.6±0.2
Beaver	9	.52±.04	.67±.06	1.7±.20	4.2±0.2
Red Squirrel	9	.53±.07	.65±.05	1.2±.18	3.1±.16
Red Fox	13	.32±.04	.46±.04	.65±.04	3.0±.56
Coyote	3	.39	.61	1.3	3.5
Marten	2	.43	.35	1.63	3.7
Porcupine	2	1.3	1.6	2.6	3.9
Mink	1	.51	.78	2.2	3.8

Values are mean or mean ± standard error.
a) Eleven animals

Q_{10} Values for Caudal Nerve Excitability

Group	Temperature Interval		
	5° to 15°	15° to 25°	25° to 35°
Muskrats (outdoor)	1.76	2.62	1.76
Muskrats (indoor)	2.88	1.80	1.92
Beaver	2.47	2.54	1.29
Red Squirrel	2.58	1.84	1.23
Red Fox	4.62	1.41	1.44
Coyote	2.69	2.13	1.56
Marten	2.27	4.65	.81
Porcupine	1.50	1.62	1.23
Mink	1.73	1.82	1.53

TABLE 14. Tibial Nerve Excitability Threshold (Volts) as Related to Temperature, 5.8 msec Stimulus Duration.

Group	No.	Temperature			
		35°	25°	15°	5°
Muskrat	7	.14±.03	.13±.03	.17±.03	.22±.04
Beaver	5	.39±.07	.36±.06	.38±.06	.46[a]±.07
Red Fox	7	.052±.008	.105±.031	.085±.025	.14[b]±.034
Coyote	3	.032	.085	.057	.33
Porcupine	1	.066	.15	.20	---
Moose	1	.15	.19	.43	.30

Values are mean ± standard error.
a) Three animals
b) Six animals

Q_{10} Values for Tibial Nerve Excitability

Group	Temperature Interval		
	5° to 15°	15° to 25°	25° to 35°
Muskrat	1.29	1.31	.93
Beaver	1.21	1.06	.92
Red Fox	1.65	.81	2.02
Coyote	5.79	.67	2.66
Porcupine	---	1.33	2.27
Moose	7.0	2.26	1.27

TABLE 15. Tibial Nerve Excitability Threshold (Volts) as Related to Temperature, 1.2 msec Stimulus Duration.

Group	No.	Temperature			
		35°	25°	15°	5°
Muskrat	9	.14±.03	.14±.02	.19±.03	.34±.04
Beaver	7	.34±.06	.35±.06	.38±.05	.71±.15
Red Fox	9	.045±.01	.087±.026	.077±.019	.17[a]±.03
Coyote	3	.036	.09	.07	.55
Porcupine	1	.09	.17	.22	1.5
Moose	1	.21	.22	.46	.40

Values are mean ± standard error.
a) Seven animals

Q_{10} Values for Tibial Nerve Excitability

Group	Temperature Interval		
	5° to 15°	15° to 25°	25° to 35°
Muskrat	1.79	1.36	1.00
Beaver	1.87	1.08	1.03
Red Fox	2.21	.88	1.93
Coyote	7.86	.77	2.50
Porcupine	6.82	1.29	1.89
Moose	.87	2.09	1.05

TABLE 16. Tibial Nerve Excitability Threshold (Volts) as Related to Temperature, .17 msec Stimulus Duration.

Group	No.	Temperature			
		35°	25°	15°	5°
Muskrat	9	.23±.04	.28±.04	.42±.05	1.16±.27
Beaver	7	.50±.05	.55±.05	.68±.04	2.2±.26
Red Fox	9	.097±.020	.180±.040	.23±.06	.77[a]±.25
Coyote	3	.078	.21	.30	2.4
Porcupine	1	.26	.36	.52	3.6
Moose	1	.22	.41	.79	1.05

Values are mean ± standard error.
a) Seven animals

Q_{10} Values for Tibial Nerve Excitability

Group	Temperature Interval		
	5° to 15°	15° to 25°	25° to 35°
Muskrat	2.76	1.50	1.22
Beaver	3.23	1.23	1.10
Red Fox	3.35	1.28	1.86
Coyote	8.00	1.43	2.69
Porcupine	6.92	1.44	1.38
Moose	1.33	1.93	1.86

TABLE 17. Tibial Nerve Excitability Threshold (Volts) as Related to Temperature, .03 msec Stimulus Duration.

Group	No.	Temperature			
		35°	25°	15°	5°
Muskrat	9	.51±.08	.75±.17	1.6±.37	4.3±.45
Beaver	7	1.11±.19	2.3±.27	3.1±.21	6.4±.41
Red Fox	9	.36±.04	.52±.08	.76±.16	3.0[a]±.56
Coyote	3	.35	.58	1.0	6.7
Porcupine	1	.61	1.0	2.5	---
Moose	1	.80	1.05	2.7	4.6

Values are mean ± standard error.
a) Seven animals

Q_{10} Values for Tibial Nerve Excitability

Group	Temperature Interval		
	5° to 15°	15° to 25°	25° to 35°
Muskrat	2.69	2.13	1.47
Beaver	2.06	1.35	2.07
Red Fox	3.95	1.46	1.44
Coyote	6.70	1.72	1.66
Porcupine	---	2.50	1.64
Moose	1.70	2.57	1.31

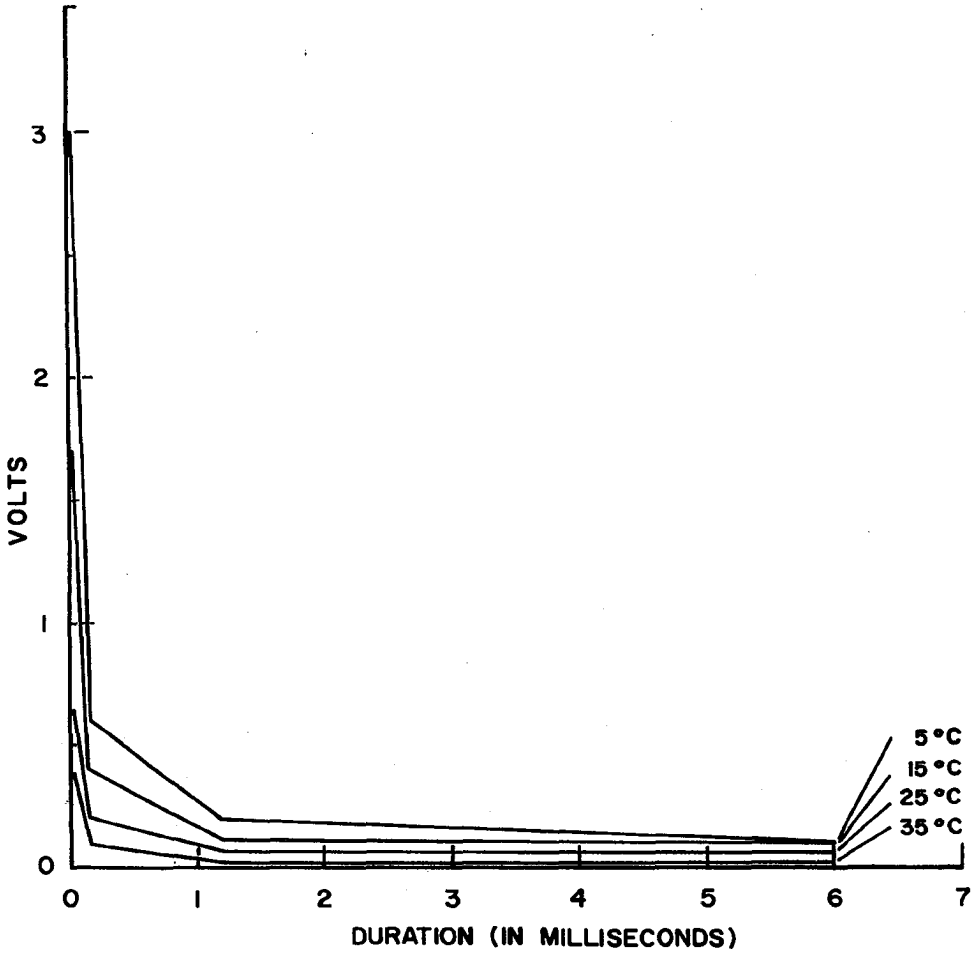

Fig. 13. Caudal nerve strength - duration curves at different temperatures; muskrat.

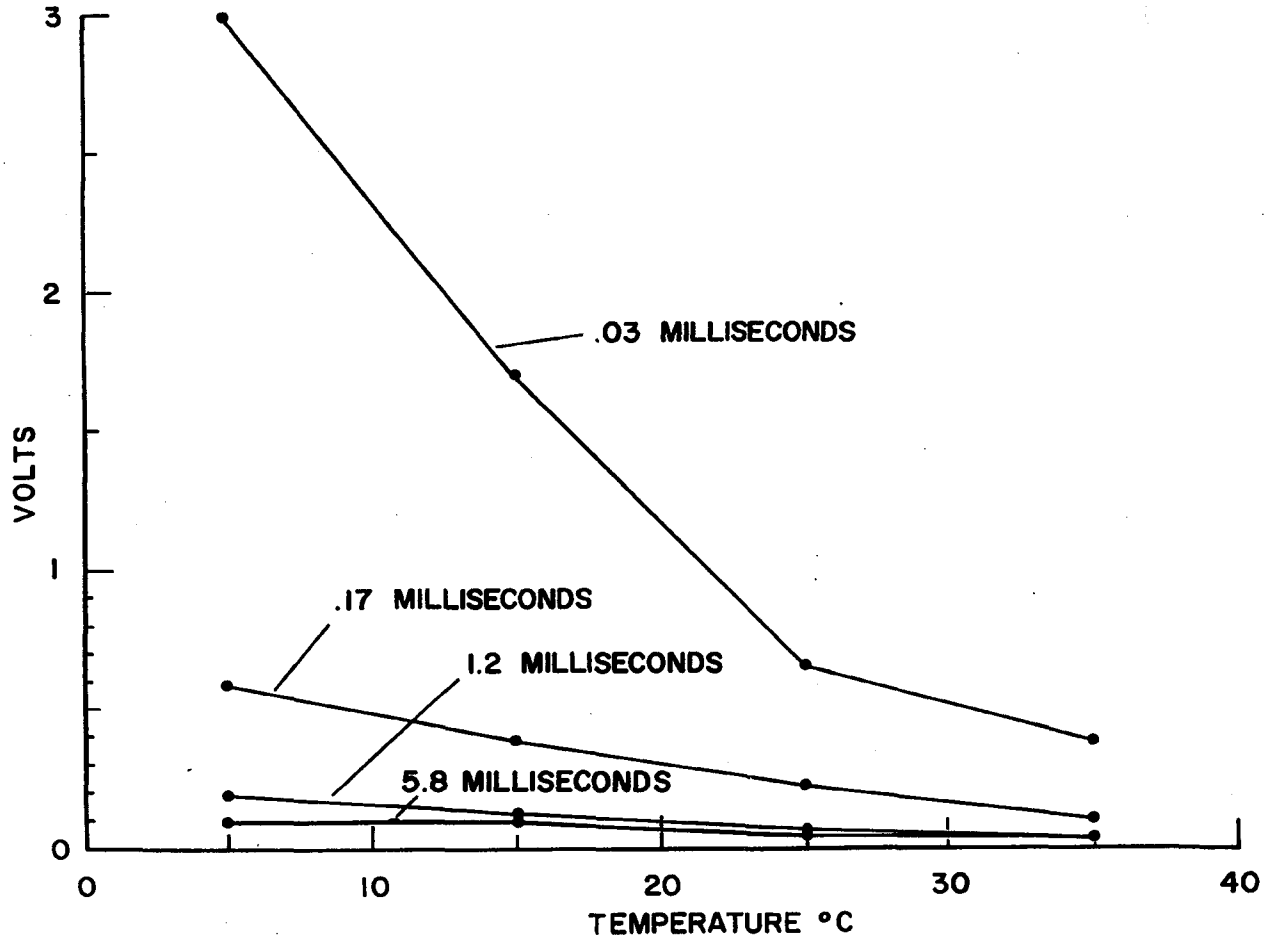

Fig. 14. Muskrat caudal nerve excitability as related to temperature and stimulus duration.

clearly shown in Figure 14 for outdoor muskrats and holds for all species examined. It should be noted that excitability threshold is reported in Tables 10 through 17 as the actual voltage required for nerve excitation, while the Q_{10} values are calculated with reference to excitability. Excitability is defined as the reciprocal of excitability threshold. There did not appear to be any consistent differences between caudal, tibial, and phrenic nerves with respect to excitability at different temperatures.

An attempt was made to measure overall nerve excitability, rather than just fast fiber excitability, in some groups by determining the stimulus increase required to go from fast fiber threshold to full development of the action potential. To the writer's knowledge, such a technique has not been previously used in studies of peripheral nerve function. Used concomitantly with the usual fast fiber excitability measurements, the technique should provide qualitative information on general fiber composition of whole nerve and, in the present case, some idea of temperature effects on smaller diameter fibers. These data are listed in Table 18.

Using the above technique it was seen that threshold responses to longer acting stimuli (1.2 milliseconds) were much less affected by temperature than when

TABLE 18. Stimulus Voltage Increase Between Threshold and Full Size Action Potential, Caudal Nerve.

Group	No.	Temperature 35°	25°	15°	5°
1.2 msec Stim. Dur.					
1964 Alaska Muskrats	13	.15 (.42-.05)	.16 (.50-.04)	.18 (.30-.04)	.24 (.37-.17)
Beaver	9	.40 (.61-.28)	.46 (.78-.35)	.42 (.59-.29)	.72 (2.2-.33)
Red Squirrel	10	.27 (.70-.06)	.35 (.80-.07)	.38 (.70-.22)	.40 (.66-.15)
Red Fox	13	.35 (.63-.07)	.43 (.74-.09)	.34[a] (.51-.20)	.47[b] (.60-.25)
.17 msec Stim. Dur.					
1964 Alaska Muskrats	13	.31 (.51-.08)	.27 (.51-.16)	.28 (.40-.19)	.64 (2.5-.35)
Beaver	9	.52 (.75-.28)	.55 (.80-.43)	.69 (.88-.44)	3.0 (4.0-2.5)
Red Squirrel	10	.38 (.65-.22)	.38 (.62-.23)	.49 (.80-.31)	1.7 (3.1-0.5)
Red Fox	13	.47 (.87-.20)	.49 (.80-.16)	.53[a] (.81-.39)	1.8[b] (3.2-0.7)
0.03 msec Stim. Dur.					
1964 Alaska Muskrats	13	.75 (3.2-.42)	.83 (2.4-.45)	1.7 (2.8-.5)	2.6 (4.6-1.9)
Beaver	9	2.5 (3.7-0.6)	3.5 (4.6-2.3)	3.5 (4.4-2.8)	5.4 (8.0-3.9)
Red Squirrel	10	1.3 (3.6-0.3)	1.7 (2.8-0.5)	2.9 (4.8-1.5)	3.8 (5.6-2.6)
Red Fox	13	.82 (2.20-.42)	1.7 (3.5-0.5)	2.6[a] (4.0-0.6)	5[b] (10-2)

Values are mean with range in parenthesis.
a) Six animals
b) Three animals

short stimuli (.03 milliseconds) were used. In other words, excitability of whole nerve followed the pattern exhibited by the fast fiber components.

The results of absolute refractory period determinations are listed in Tables 19, 20, and 21. Caudal nerves of the various species show similar values at all temperatures, except for the apparent deviation of the coyote and porcupine at 15° and 5°C. Absolute refractory periods for these two animals are lengthened at the lower temperatures. Comparison of indoor and outdoor muskrats indicates a significant increase in refractory period at temperatures above 5°C in the indoor muskrat caudal nerves.

In the instances of tibial nerves remaining functional at 5°C, three of the four species showed significant increases in refractory periods at lower temperatures (15° and 5°C), compared with values obtained for caudal nerves. Absolute refractory period as a function of temperature is plotted for caudal, tibial, and phrenic nerves of beaver in Figure 15. It is evident that refractory periods at low temperatures are greater in the tibial than in the caudal nerves and are still greater in the phrenic than in the tibial. If refractory periods are converted into maximum nerve conduction frequency

TABLE 19. Caudal Nerve Absolute Refractory Period as Related to Temperature.

Group	No.	Temperature 35°	25°	15°	5°
Muskrats (outdoor)	14	.26±.04	.70±.02	1.7±.05	10.4±.14
Muskrats (indoor)	5	.44±.04	.88±.10	2.2±.03	10.5±1.1
Beaver	9	.34±.06	.72±.04	1.9±.20	10.1±1.1
Red Squirrel	9	.34±.07	.65±.07	2.4±.4	11.1±.7
Red Fox	13	.27±.03	.82±.09	2.5±.3	12.7±3.5[a]
Coyote	3	.30	.67	2.8	21
Marten	2	.17	.98	2.0	11.3
Porcupine	2	.38	1.1	5.0	---
Mink	1	.24	.65	2.3	11.0

Values are mean ± standard error.
a) Nine animals

Statistical Results

Comparison	Temperature 35°	25°	15°	5°
Indoor vs Outdoor Muskrats	P=.025	P<.025	P<.001	N.S.

Q_{10} Values

Group	Temperature Interval 5°-15°	15°-25°	25°-35°
Muskrats (outdoor)	5.12	2.43	2.69
Muskrats (indoor)	4.77	2.50	2.00
Beaver	5.32	2.63	2.12
Red Squirrel	4.62	3.69	1.91
Red Fox	5.08	3.05	3.04
Coyote	7.50	4.18	2.23

TABLE 20. Tibial Nerve Absolute Refractory Period as Related to Temperature.

Group	No.	Temperature			
		35°	25°	15°	5°
Muskrats (outdoor)	9	.37±.06[a]	.71±.09	3.0±.24	18±1.5
Beaver	7	.26±.05	.95±.12	4.0±.1	18±2.1
Red Fox	9	.22±.03	.83±.10	2.6±.5	10±1.7[a]
Coyote	3	.15	.68	3.5	---
Porcupine	1	.30	.76	4.0	---
Moose	1	.20	.73	2.2	16

Values are mean ± standard error.
a) Six animals

Q_{10} Values

Group	Temperature Interval		
	5° to 15°	15° to 25°	25° to 35°
Muskrats (outdoor)	6.00	4.22	1.92
Beaver	4.50	4.21	3.65
Red Fox	3.85	3.13	3.77
Moose	7.27	3.01	3.65

TABLE 21. Alaska Beaver; Phrenic Nerve Absolute Refractory Period as Related to Temperature.

No.	Temperature			
	35°	25°	15°	5°
6	.47±.06	1.25±.32	6.4±.07	36±1.4

Q_{10} Values

Temperature Interval		
5° to 15°	15° to 25°	25° to 35°
5.62	5.12	2.65

Statistical Evaluation, Beaver Nerve Absolute Refractory Periods.

Comparison	Temperature			
	35°	25°	15°	5°
Tibial vs Caudal	N.S.	N.S.	P<<.001	P<.005
Phrenic vs Caudal	N.S.	P<.05	P<<.001	P<<.001
Phrenic vs Tibial	P=.03	N.S.	P<<.001	P<<.001

N.S.-Not Significant

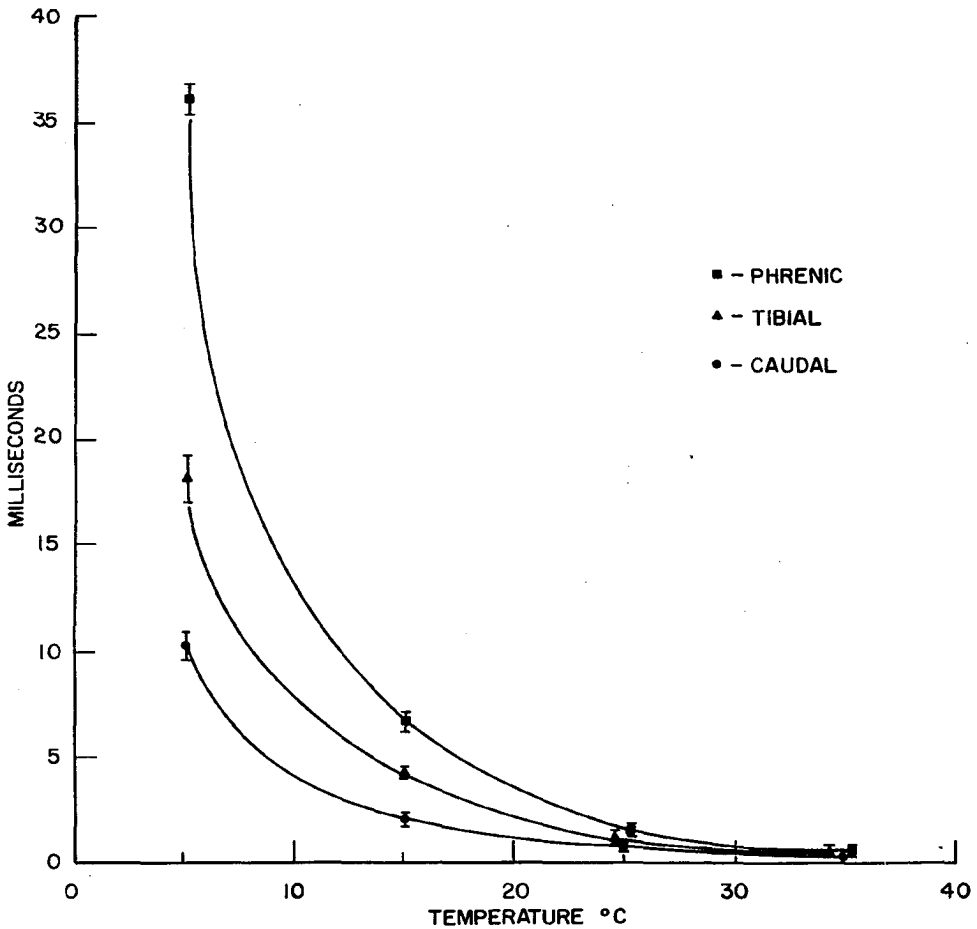

Fig. 15. Absolute refractory period as related to temperature; beaver peripheral nerve comparisons.

$\left(\frac{1{,}000 \text{ msec/sec}}{\text{refractory period in msec}}\right.$ = maximum impulse frequency per second, fast fibers), the following values are obtained for the different beaver nerves:

Nerve	35°C	5°C	Decrease Factor $\left(\frac{35° \text{ value}}{5° \text{ value}}\right)$
Caudal	2950*	99*	30
Tibial	3850	56	69
Phrenic	2130	28	76

* Impulses per second.

The above values for impulse conduction are theoretical maxima and are probably far from attainable by the majority of fibers in the living animal. If the limiting values at each temperature are assumed to be about half of those given, the maximum impulse frequency for caudal nerve at 5°C would be about 50 impulses per second; probably a borderline value for most fibers carrying messages. The values for tibial and phrenic nerve at 5°C would be 28 and 14 impulses, respectively, which would undoubtedly be below the limit at which normal transmission of some peripheral signals could take place.

Values calculated for absolute refractory period Q_{10} show a noticeable, though somewhat variable, decrease in rate function with increasing temperature. As might be anticipated from inspection of Figure 15, Q_{10} values

for tibial and, more especially, phrenic nerves are higher than for caudal nerves in the 15° to 25°C temperature region.

The gross action potential characteristics determined for each temperature included both duration and magnitude. Durations are given in Tables 22, 23, and 24. Again, the well insulated red fox shows functional changes in caudal nerve at lower temperature that are out of proportion to the responses of other animals. The compound action potentials for fox, porcupine, and coyote caudal nerves showed durations of about twice those of other animals at 5°C, although action potential durations for all species at 35°C were very similar. Action potential durations for muskrat caudal and tibial nerves were similar. Red fox tibial nerve action potentials showed increased durations at lower temperatures compared with muskrat tibial nerves and were similar to red fox caudal nerves. Beaver tibial nerves were intermediate in character. Beaver phrenic nerves, compared with caudal and tibial nerves, showed a marked increase in action potential duration when cooled from 15° to 5°C, with a Q_{10} in this temperature range averaging 6.8. A comparison of action potential duration in caudal, tibial, and phrenic nerves of beaver is made in Figure 16.

TABLE 22. Caudal Nerve Action Potential Duration (milliseconds) as Related to Temperature.

Group	No.	Temperature 35°	25°	15°	5°
Muskrats (outdoor)	13	1.2 (1.6-0.9)	1.9 (2.2-1.5)	3.8 (4.6-3.0)	11.3 (13.0-9.0)
Beaver	9	1.1 (1.5-0.9)	1.8 (2.2-1.6)	3.4 (4.6-3.1)	11.7 (15.0-10.0)
Red Squirrel	9	0.8 (1.4-0.6)	1.5 (2.4-1.1)	3.1 (4.0-2.5)	9.0 (13.0-7.6)
Red Fox	13	1.5 (1.8-1.2)	2.7 (3.0-2.3)	5.7[a] (6.0-5.2)	23[a] (35-9)
Coyote	3	1.3 (1.4-1.2)	2.8 (3.0-2.4)	4.7 (4.8-4.6)	19 (28-13)
Marten	2	1.4 (1.4-1.3)	1.9 (2.0-1.8)	3.8 (4.0-3.6)	10.5 (11-10)
Porcupine	2	1.0 (1.2-0.7)	2.3 (2.8-1.8)	4.4 (5.5-3.2)	25

Values are mean with range in parenthesis.
a) Five animals

Q_{10} Values

Group	Temperature Interval 5° to 15°	15° to 25°	25° to 35°
Muskrats (outdoor)	3.0 (4.1-2.6)	2.0 (2.6-1.7)	1.6 (1.8-1.4)
Beaver	3.3 (4.2-2.8)	2.1 (2.6-1.8)	1.6 (2.0-1.3)
Red Squirrel	2.9 (3.6-2.5)	2.2 (2.8-1.5)	1.9 (2.4-1.4)
Red Fox	3.5[a] (5.8-1.6)	2.3[a] (2.5-1.9)	1.8 (2.5-1.5)
Coyote	3.9 (5.8-2.8)	1.7 (1.9-1.6)	2.2 (2.5-1.8)
Marten	2.8	2.0	1.4
Porcupine	7.8	1.9	2.4

TABLE 23. Tibial Nerve Action Potential Duration (milliseconds) as Related to Temperature.

Group	No.	Temperature			
		35°	25°	15°	5°
Muskrats (outdoor)	9	0.8 (1.0-0.7)	1.4 (1.8-1.2)	2.8 (3.6-2.4)	10.0 (18-7.2)
Beaver	7	1.2 (1.5-0.8)	2.2 (3.0-1.4)	4.2 (7.2-2.8)	14.9 (25-7.2)
Red Fox	8	1.3 (1.6-0.9)	2.2 (2.6-1.6)	4.2[a] (4.4-4.0)	19 (20-18)

Values are mean with range in parenthesis.
a) Five animals

Q_{10} Values

Group	Temperature Interval		
	5° to 15°	15° to 25°	25° to 35°
Muskrats (outdoor)	3.8 (6.9-3.3)	2.0 (2.4-1.8)	1.7 (2.0-1.5)
Beaver	3.6 (5.7-2.3)	1.9 (2.4-1.3)	1.9 (2.3-1.3)
Red Fox	4.5[a] (4.5-4.4)	2.5[a] (2.8-2.2)	1.7 (1.9-1.5)

Values are mean with range in parenthesis.
a) Five animals

TABLE 24. Beaver Phrenic Nerve Action Potential Duration as Related to Temperature.

No.	Temperature			
	35°	25°	15°	5°
6	0.9 (1.3-0.6)	2.3 (3.2-1.0)	5.2 (7.2-3.0)	33.9 (50-17.5)

Q_{10} Values

Temperature Interval		
5° to 15°	15° to 25°	25° to 35°
6.8	2.3	2.5

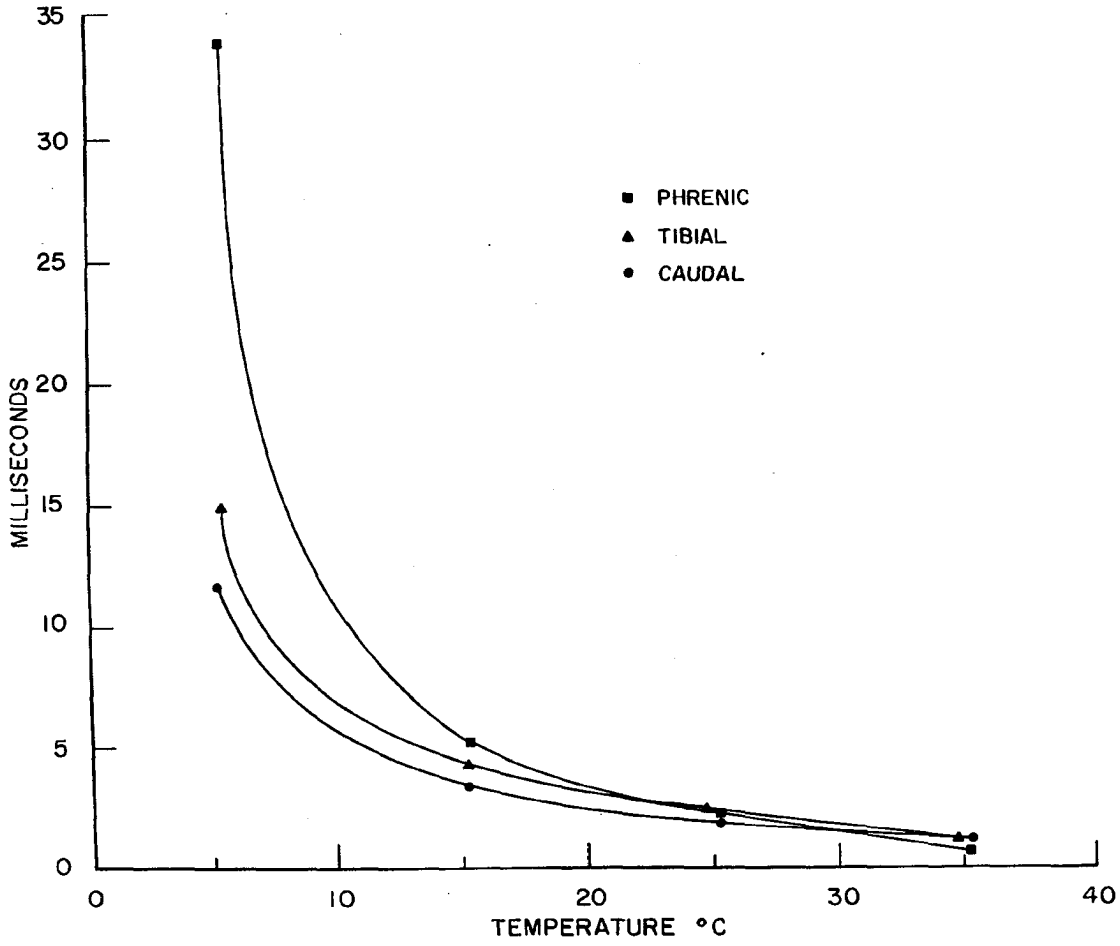

Fig. 16. Action potential duration as related to temperature in beaver peripheral nerves.

Action potential duration may also be used to give an estimate of maximal nerve conduction frequency, since any given point on the nerve through which an action potential passes remains refractory to the passage of additional impulses for a portion of the action potential duration. It is necessary to make the somewhat error-prone assumption that duration of the whole nerve action potential is not greatly different from the duration of an individual fiber action potential. If two-thirds of the action potential duration is taken as a reasonable estimate of the time during which no new impulses can be conducted, maximum impulse frequency for beaver caudal nerve at 5°C would be

$$\frac{1{,}000 \text{ msec/sec}}{2/3(12 \text{ msec. action potential duration})} = 125 \text{ impulses/sec.}$$

Maximum impulse frequency for beaver tibial nerve at 5°C would be

$$\frac{1{,}000 \text{ msec/sec.}}{2/3(15 \text{ msec})} = 100 \text{ impulses/sec.}$$

For beaver phrenic nerve at 5°C, maximum impulses frequency would be

$$\frac{1{,}000 \text{ msec/sec}}{2/3(34 \text{ msec})} = 45 \text{ impulses/sec.}$$

All of these values for maximum impulse conduction are somewhat higher than values calculated from absolute refractory periods but are well within the limits of

error involved in the various assumptions and estimations used in making the calculations.

Action potential magnitudes at various temperatures are given in Table 25. Due to variations in recording distance, in addition to the effect of other variables previously discussed, only gross differences in action potential magnitude need be considered, and even these results must be interpreted with caution. Magnitudes were consistently greatest at 25° and 15°C and decreased at both higher and lower temperatures. As a rule greater decreases in magnitude occurred with smaller temperature changes as the temperature was approached at which a nerve ceased conducting. These decreases show up at the 5°C temperature in fox caudal nerve, fox and coyote tibial nerve, and beaver phrenic nerve. Other nerves do not show such a marked magnitude drop at 5°C.

Alaska-Louisiana Muskrat Comparisons

Comparison of a group of twelve Southern Louisiana muskrats with eleven muskrats from the Fairbanks area was accomplished during a five month period from May to October, 1963. Muskrats from Louisiana were obtained in late spring, while the Alaskan animals were captured during spring and summer. Only ventral caudal nerves were tested.

TABLE 25. Peripheral Nerve Action Potential Magnitude (mv) as Related to Temperature.

Group	No.	Temperature 35°	25°	15°	5°
A. Caudal Nerve					
Muskrat (outdoor)	15	2.4	3.1	3.9	3.7
Muskrat (indoor)	5	4.2	5.1	5.3	3.8
Beaver	9	1.7	2.0	2.5	2.3
Red Squirrel	9	3.5	4.4	3.6	3.1
Red Fox	6	3.0	4.6	3.9	0.8
Coyote	3	1.9	2.7	4.2	2.0
B. Tibial Nerve					
Muskrat (outdoor)	9	4.4	5.5	5.4	3.0
Beaver	7	2.7	3.3	3.7	2.4
Coyote	3	3.3	4.0	3.8	0.8
Red Fox	3	4.1	5.0	4.4	0.7
C. Phrenic Nerve					
Beaver	6	5.7	6.4	5.5	1.3

All values are mean.

Use of a plexiglas nerve chamber immersed in a water bath and ice-salt mixtures to obtain below freezing temperatures resulted in freezing and action potential extinction point values higher than those obtained in later studies of muskrats and other animals using the metal nerve chamber. The average temperature of action potential disappearance in Alaska muskrats was -3.0°C, while the average temperature of disappearance for Louisiana muskrats was -2.0°C. It is doubtful that the difference between these two means is of significance due to the amount of variation between individuals and, perhaps more important, due to improved dissection and handling technique after the first several animals (which were Louisiana muskrats). Statistically, if one assumes proper technique in data collection, the temperatures for action potential disappearance are lower in the Alaska muskrats only at the probability level of $P<.06$.

Conduction velocity, excitability, absolute refractory period, and action potential magnitude for the two groups are compared in Table 26. Statistical analysis is given in Table 27. Conduction velocity was significantly greater in Louisiana muskrat nerve at all temperatures, showing the greatest increase over values for Alaskan animals at the higher temperatures. This feature is more clearly seen in Figure 17. The Q_{10} values for various caudal nerve characteristics are given in Table 28.

TABLE 26. Alaska and Louisiana Muskrats. Caudal Nerve Function as Related to Temperature.

Group	No.	Temperature (°C) 35°	25°	15°	5°
Conduction Velocity (M/sec)					
Alaska	11	36.9±2.5	26.6±2.0	17.9±.9	9.1±.6
Louisiana	12	42.8±1.1	30.7±.7	19.4±.6	9.8±.2
Excitability Threshold (Volts) 1.2msec Stimulus Duration					
Alaska	11	.024±.006	.031±.007	.111±.006	.25±.008
Louisiana	12	.025±.008	.036±.006	.15±.02	.28±.02
0.17msec Stimulus Duration					
Alaska	11	.075±.017	.13±.033	.39±.022	.63±.03
Louisiana	12	.12±.03	.20±.03	.55±.05	1.2±.24
0.03msec Stimulus Duration					
Alaska	11	.34±.053	.51±.076	1.32±.22	3.5±.06
Louisiana	12	.47±.20	.76±.14	2.8±.4	5.2±.5

Values are mean ± standard error.

TABLE 26 (Cont.). Alaska and Louisiana Muskrats. Caudal Nerve Function as Related to Temperature.

Group	No.	Temperature (°C)			
		35°	25°	15°	5°
Absolute Refractory Period (msec)					
Alaska	11	.56±.03	1.0±.07	1.9±.1	10±.4
Louisiana	12	.60±.03	1.0±.05	2.3±.15	10.2±.24
Action Potential Magnitude (mv)					
Alaska	11	4.3	4.8	6.0	4.6
Louisiana	12	4.8	5.7	6.5	4.6

Values are mean ± standard error.

TABLE 27. Statistical Results, Alaska-Louisiana Muskrat Nerve Function. Group Mean Comparisons.

Function	Temperature (°C)			
	35°	25°	15°	5°
Conduction Velocity	P<.001	P<.001	P=.03	P=.03
Excitability, 1.2 msec Stimulus Duration	N.S.*	N.S.	N.S.	N.S.
Excitability, .17 msec Stimulus Duration	N.S.	N.S.	P .01	P<<.001
Excitability, .03 msec Stimulus Duration	N.S.	N.S.	P<.01	P<.01
Absolute Refractory Period	N.S.	N.S.	P=.05	N.S.

* N.S.=No significant difference between means.

TABLE 28. Q_{10} Values for Various Caudal Nerve Functions, Alaska and Louisiana Muskrats.

Group	Temperature Interval		
	5° to 15°	15° to 25°	25° to 35°
Conduction Velocity			
Alaska	1.98	1.48	1.40
Louisiana	1.98	1.56	1.39
Excitability			
Alaska	2.25	3.58	1.29
Louisiana	1.87	4.17	1.44
Alaska	1.62	3.00	1.73
Louisiana	2.18	2.75	1.67
Alaska	2.65	2.59	1.50
Louisiana	1.85	3.68	1.62
Absolute Refractory Period			
Alaska	5.26	1.90	1.78
Louisiana	4.43	2.30	1.67

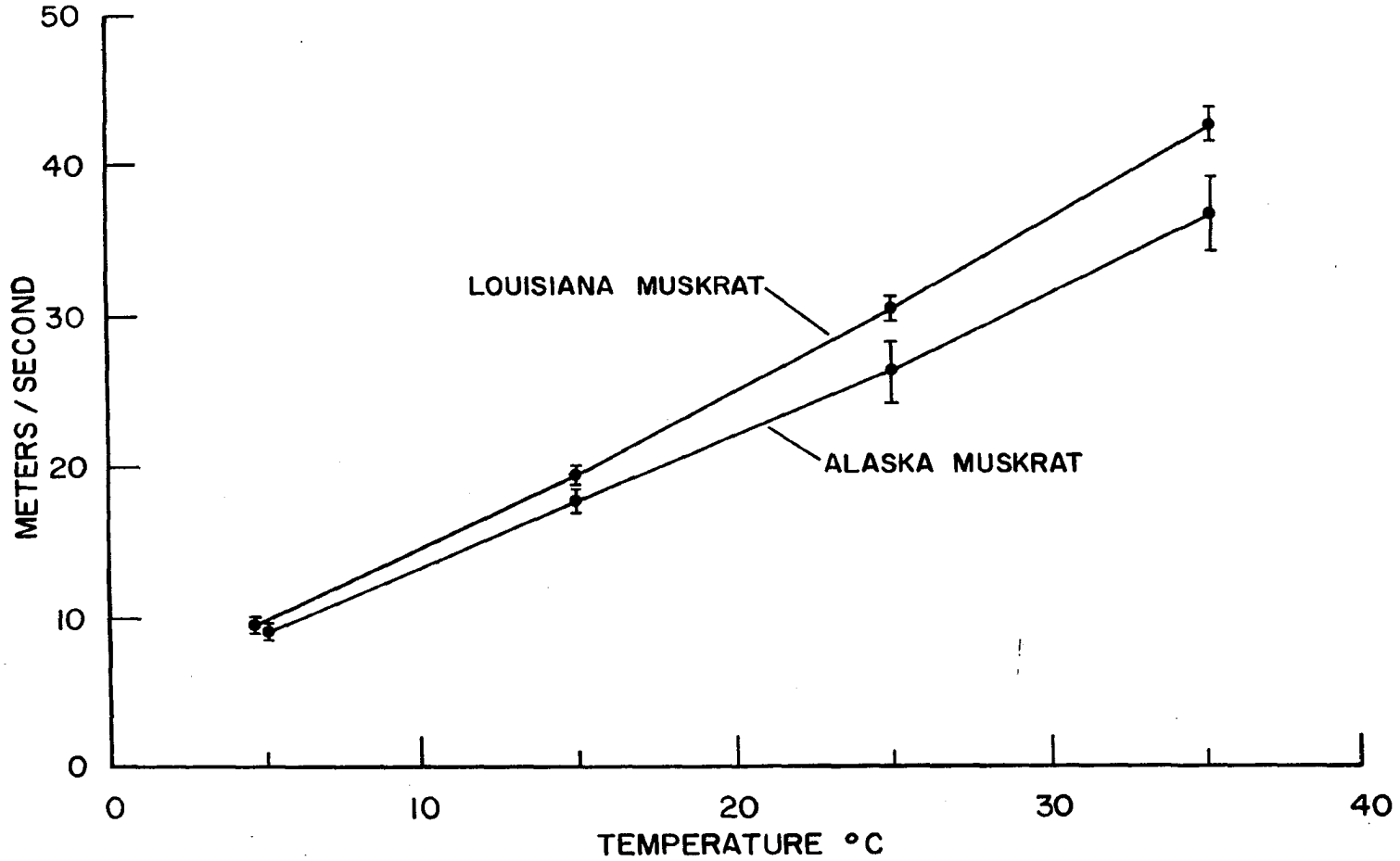

Fig. 17. Caudal nerve conduction velocity as related to temperature. Alaska and Louisiana muskrats.

Excitability differences between the two groups of muskrats were not apparent at any temperatures when using stimuli of longer duration (1.2 milliseconds). However, with short duration stimuli, increased stimulus strength was required to excite the Louisiana muskrat nerves at lower temperatures, and some indication of an excitability difference is apparent at higher temperatures.

No consistent temperature-related differences could be seen between Alaska and Louisiana nerves with respect to absolute refractory periods, nor were there any gross differences in action potential appearance and magnitude.

Nerve Responses to Supercooling

Caudal nerves in five of the eight species examined remained excitable and conducted action potentials at temperatures well below their expected freezing point. In those instances where nerve temperature was allowed to drop below -5°C, freezing, accompanied by spontaneous rewarming (SR), occurred in the great majority of nerves. The spontaneous rewarming, as measured by the nerve chamber thermocouples, was carefully followed in a number of cases. Spontaneous rewarming characteristics varied to some extent with rate of prior cooling, with the temperatue at which freezing occurred, and with the type of nerve

used. Some of the spontaneous rewarming characteristics due to freezing of supercooled nerve are given in Table 29.

For the nerves tested in the metal nerve chamber, the mean temperature at onset of freezing (or SR) varied only from -4.6° to -6.4°C, with a total range of -4.0° to -6.7°C. The lowest temperatures were obtained during the most recent tests on indoor muskrats in which cooling speed was greater, especially at the lower supercooling temperatures, than for previous animals tested. The lower temperature at onset of freezing altered the rate of SR, causing SR to reach a peak much more rapidly. This combination of factors also resulted in a greater temperature rise during SR in the indoor muskrats. Otherwise, variations in magnitude of SR are not considered to be species related to any important extent.

Spontaneous rewarming of supercooled nerves first came to notice as a result of the striking action potential changes that occurred at such time. The rewarming was accompanied by a marked increase in action potential amplitude (Figure 19), waveform changes, and an increase in conduction velocity. Figure 18 shows a typical spontaneous rewarming curve obtained following moderately slow supercooling of a red squirrel caudal nerve. In this

TABLE 29. Some Responses Associated With Spontaneous Rewarming (SR) Due to Freezing in Supercooled Caudal Nerves.

Group	Nerves Showing SR	Temp. at Onset of SR (°C)	Temp. Rise During SR (°C)	AP Amplitude Increase* During SR (mv)	Conduction Velocity Increase During SR (M/sec/°C)
Plastic Nerve Chamber					
Alaska Muskrats	5	-3.6 (-2.5 to -4.5)	1.6 (1.0 to 1.8)	1.4	
Louisiana Muskrats	2	-2.8 (-2.5 to -3.0)	Not measured	---	
Metal Nerve Chamber					
Muskrats (indoor)	3	-6.4 (-5.8 to -6.7)	4.8 (4.2 to 5.3)	1.8	0.4 (1 animal)
Muskrats (outdoor)	6	-4.6 (-4.0 to -5.1)	1.9 (1.o to 3.2)	---	
Beaver	5	-5.2 (-4.6 to -5.6)	3.4 (2.0 to 4.4)	---	0.68 (1 animal)
Red Squirrel	6	-5.3 (-4.9 to -6.0)	2.9 (2.1 to 3.4)	2.8 (1.8 to 3.4)	0.5 Same in 3 animals
Marten	1	-5.0	4.0	---	
Mink	1	-5.1	Not measured	---	

Values are mean with range in parenthesis.
* AP=Action Potential. Increase is with reference to amplitude of the AP just before and at the peak of rewarming. Measurement was not possible in all cases.

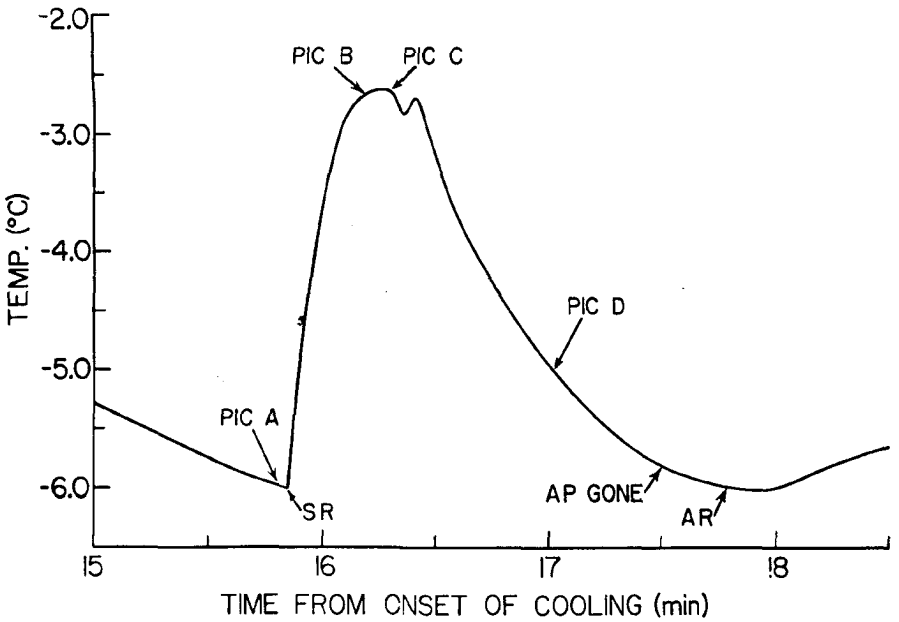

Fig. 18. Cooling curve for red squirrel caudal nerve showing spontaneous rewarming. SR; onset of spontaneous rewarming. AP gone; point at which an action potenial could no longer be elicited. AR; artificial warming begun. Pic A, B, C, and D; points at which exposures were made for action potentials shown in Fig. 19.

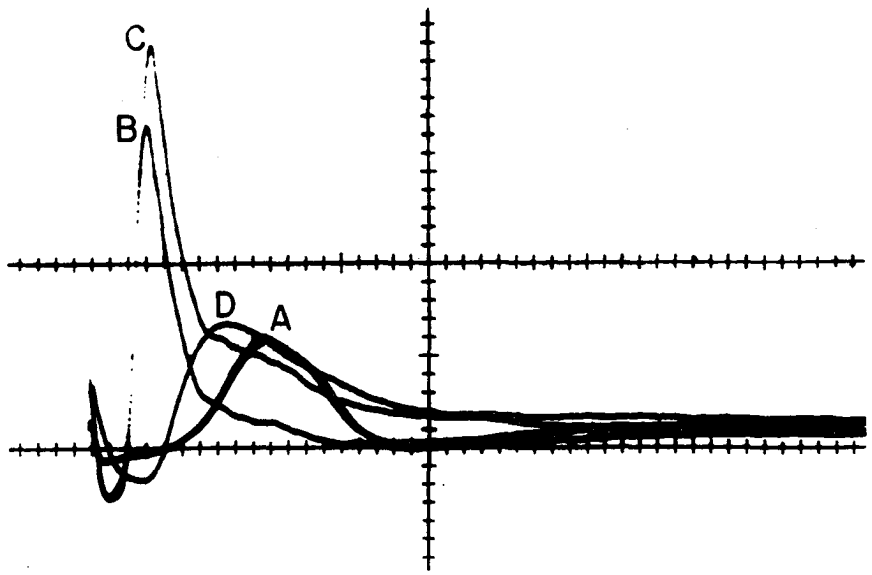

Fig. 19. Multiple exposure showing increase in height, conduction velocity, and changes in action potential wave-form with spontaneous rewarming (SR). A, just before SR, B and C, at height of SR; D, near end of SR. Oscilloscope sweep, 20 msec/cm; sensitivity, 1 mv/cm. Nerve stimulus supermaximal.

instance the temperature increase during SR was 3.3°C, and the SR reached a peak in about 25 seconds. The action potential changes that accompanied spontaneous rewarming may be seen in Figure 19. Action potential magnitude increased from 1.2 millivolts (A) to 4.3 millivolts (C), while conduction velocity increased from 1.3 meters per second to 3.0 meters per second.

A spontaneous rewarming curve obtained following more rapid cooling of a muskrat caudal nerve is seen in Figure 20 and the the accompanying action potential changes in Figure 21. Nerve temperature reached -6.7°C before freezing occurred, but when freezing started it went to completion in approximately two seconds, resulting in the rapid temperature rise shown in Figure 20. The SR curve showed much better plateauing than in Figure 18, and the nerve chamber temperature remained above the pre-SR level for several minutes or until artificial rewarming was begun.

It is probable that a sizeable heat of fusion contribution was provided by the freezing of droplets or small puddles of Locke's solution present on the floor and walls of the nerve chamber. This contribution varied considerably from one experiment to the next but is not believed to have greatly influenced the results. Several

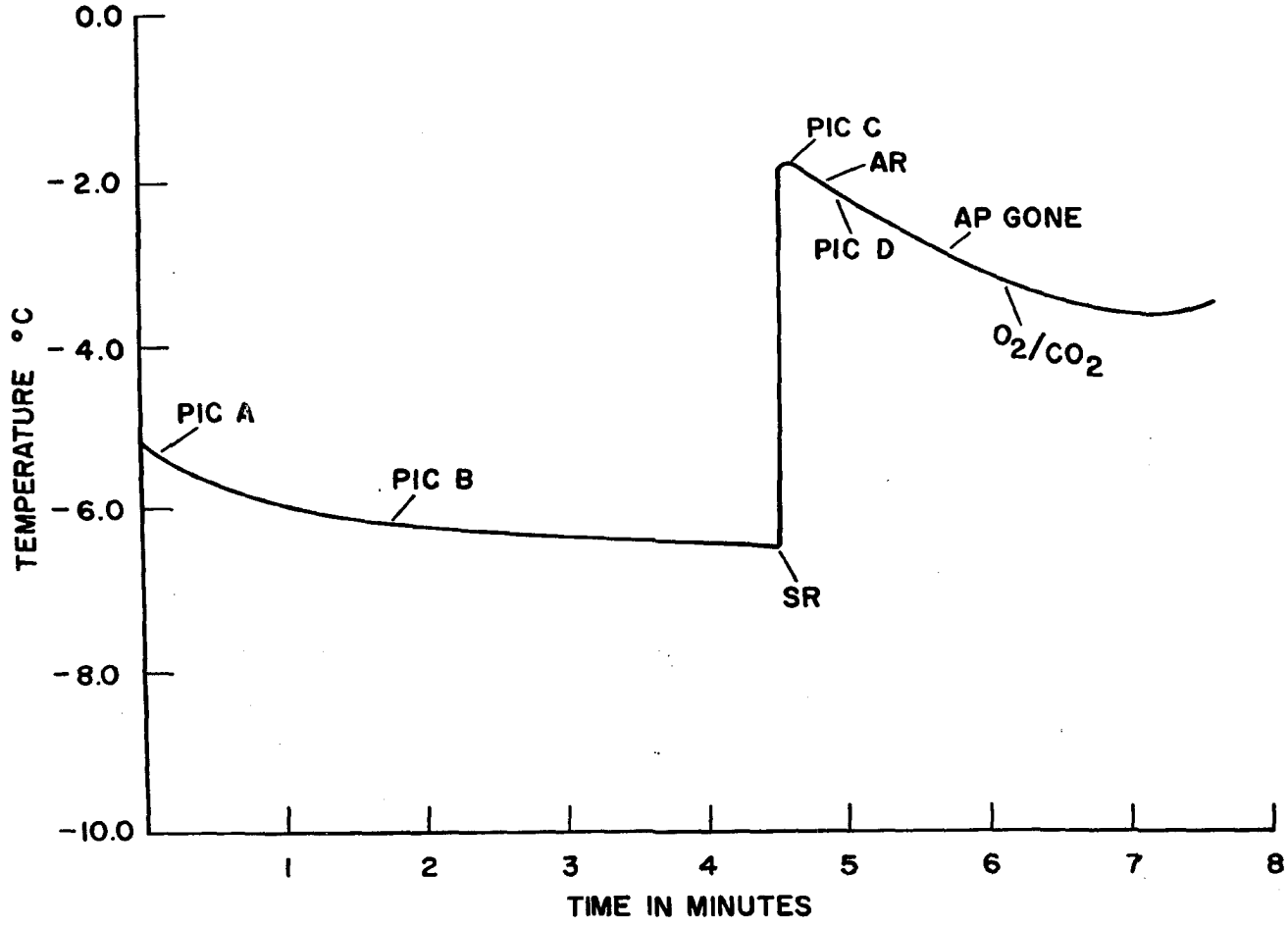

Fig. 20. Muskrat caudal nerve cooling curve, symbols same as in Fig. 18.

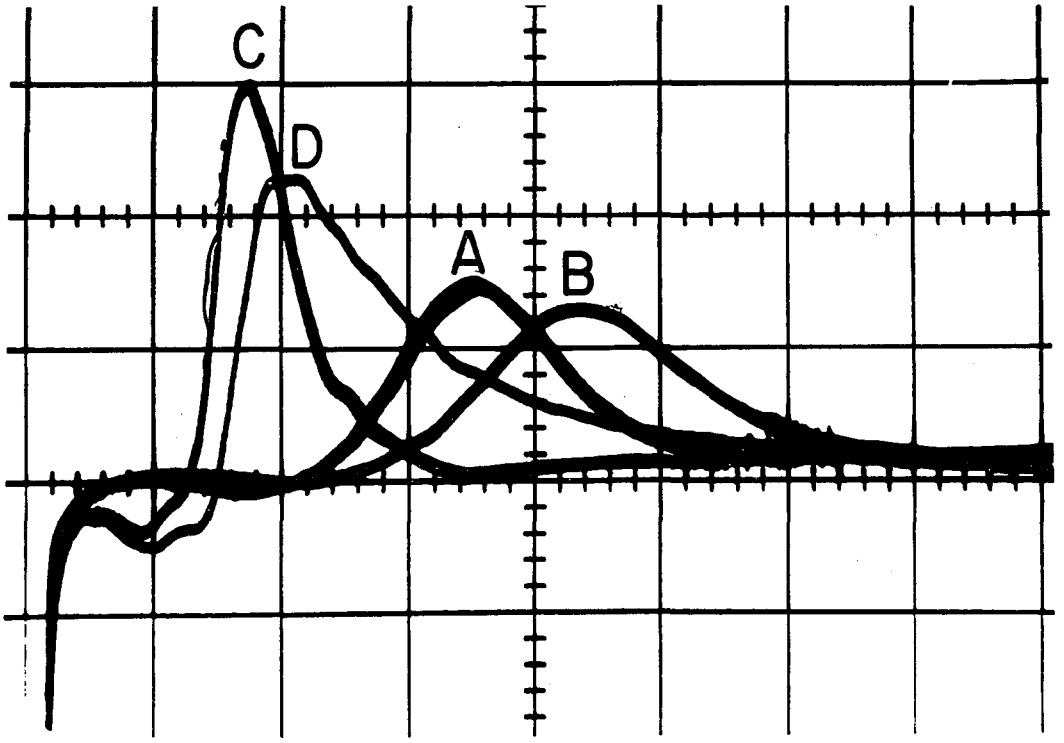

Fig. 21. Changes in action potential characteristics with spontaneous rewarming (SR). Pic A; before SR, temperature -5.3°C. Pic B; before SR, temperature -6.1°C. Pic C: at height of SR, temperature -1.8°C. Pic D; 1/2 minute after peak of SR, temperature -2.3°C.

mock experiments were made in which normal cooling procedure was followed without a nerve in the chamber, and no SR was seen between 0° and -10°C. This fact indicates that either a nerve must be present for freezing to spontaneously occur in the region of -4° to -7°C or such a small amount of Locke's solution remains adhered to the inner chamber surfaces that its freezing does not cause a measurable temperature rise.

Some approximate calculations have been made to determine if the temperature increase in the nerve chamber during freezing could reasonably be ascribed to the freezing of a small nerve, such as the red squirrel caudal nerve. It is assumed (from actual weight determinations) that the amount of water available for freezing in such a nerve would be 50 milligrams or more.

Calculations:

Heat capacity (C_p) of air at constant pressure at -5°C ... 0.240 cal/gm/°C (Lange, 1956).

Volume of nerve chamber ... (15 cm)(1.2 cm)(1.0 cm) = 18 cc or 18 ml.

Density of air at -5°C ... 13.17×10^{-4} gm/ml (Lange, 1956).

Weight of chamber air ... (18 ml)(13.17×10^{-4} gm/ml) = 2.38×10^{-2} gm.

Heat capacity of chamber air ($C_{p(air)}$) at -5°C: (weight of chamber air)($C_{p(air)}$) = 2.38 x 10^{-2} gm(.240 cal/gm/°C) = .00571 cal/°C.

Heat of fusion (ΔH_{273}) of water at 0°C ... -79.7 cal/gm (Daniels and Alberty, 1961).

Heat of fusion of water at -5°C (268° Absolute): $C_{p,\ H_2O(liq)}$ = 1 cal/deg/gm; $C_{p,\ H_2O(solid)}$ = .49 cal/deg/gm; $\Delta H_{268} = \Delta H_{273} + [C_{p,\ H_2O(solid)} - C_{p,\ H_2O(liq)}](268-273)$ = -79.7 cal/gm + (-.51 cal/deg/gm)(-5°C) = -79.7 cal/gm + 2.6 cal/gm = -77.1 cal/gm.

A typical section of nerve weighed approximately 100 milligrams. If 50 percent of this weight is available as free water, freezing of 0.05 gm. of water (in the nerve) at -5°C would release (.05 gm)(-77.1 cal/gm) = -3.85 cal.

The chamber air heat capacity was .00571 cal/°C, and chamber air temperature often increased about 3°C, so the amount of heat required to raise the temperature of the chamber air by this amount would be

(3°C)(.00571 cal/°C) = .0171 cal.

According to the preceding calculations 3.85 calories were available, so the chamber air temperature rise actually measured would be easily accounted for. This is true even if allowance is made for considerable

heat loss through the chamber walls, incomplete freezing of the nerve, and lag in the temperature measuring system.

The increase in action potential height and conduction velocity during spontaneous rewarming fell within the ranges of values that were seen at the same temperatures during early supercooling, i.e., as nerve temperature was on the decrease prior to freezing. Conduction velocities obtained near the height of spontaneous rewarming are plotted for several red squirrels and a muskrat in Figure 22a and 22b. Values obtained before spontaneous rewarming are also plotted. In the muskrat the spontaneous rewarming values fell a little below those expected. This can be attributed to a lag in the rise in nerve temperature as compared with chamber temperature. Spontaneous rewarming occurred at a slower rate in the red squirrel nerves than in the muskrat nerve (Figures 18 and 20),and the temperature rise in the latter nerve probably never reached peak chamber temperature.

Following spontaneous rewarming, artificial rewarming was initiated to raise the nerve temperature to 25°C for final testing. Reappearance of action potentials in most cases did not occur at the melting point but usually occurred at temperatures well above 0°C. To avoid further nerve deterioration, most nerves were rewarmed

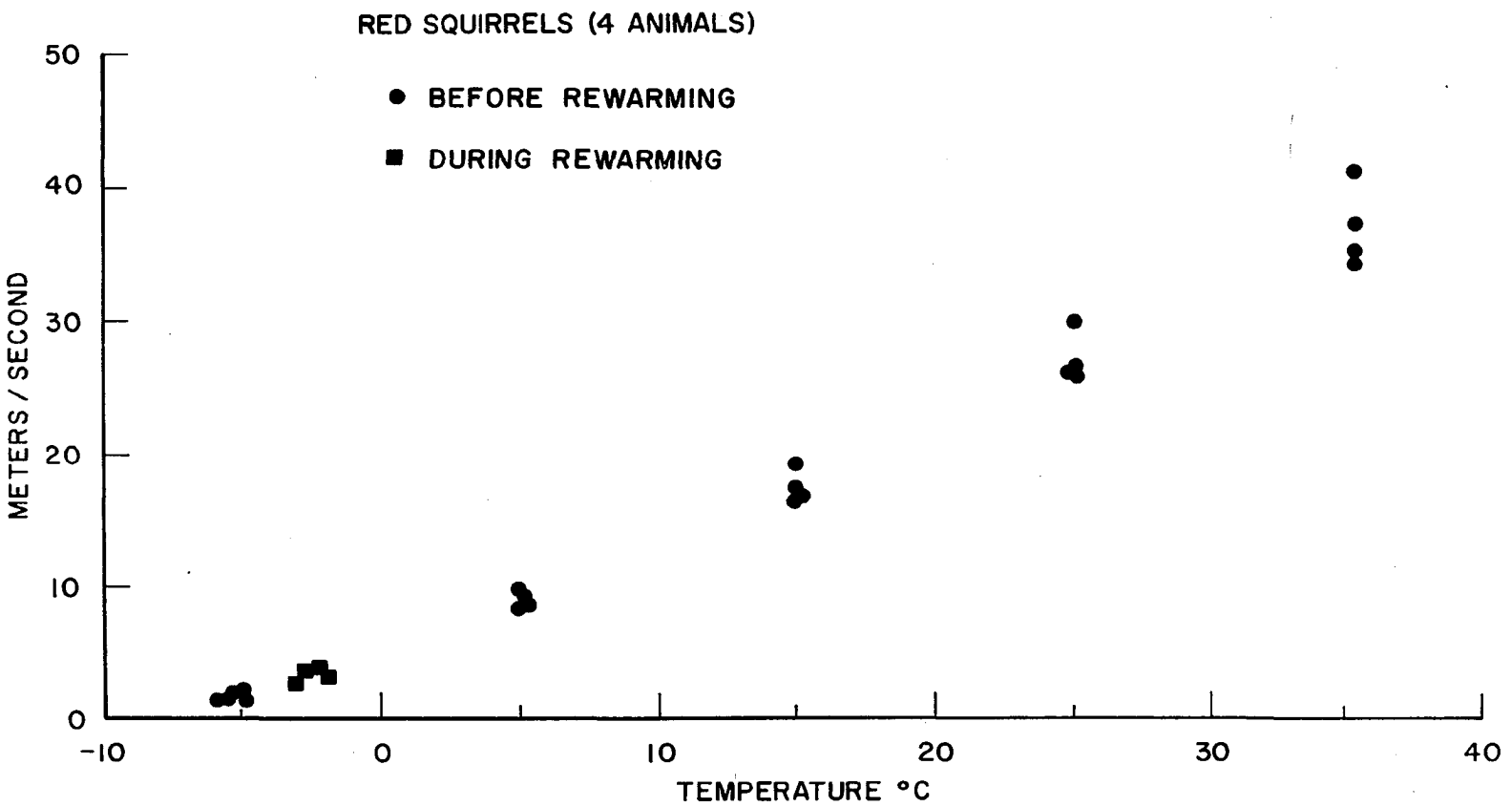

Fig. 22a. Caudal nerve conduction velocities before and during spontaneous rewarming; red squirrel (4 animals).

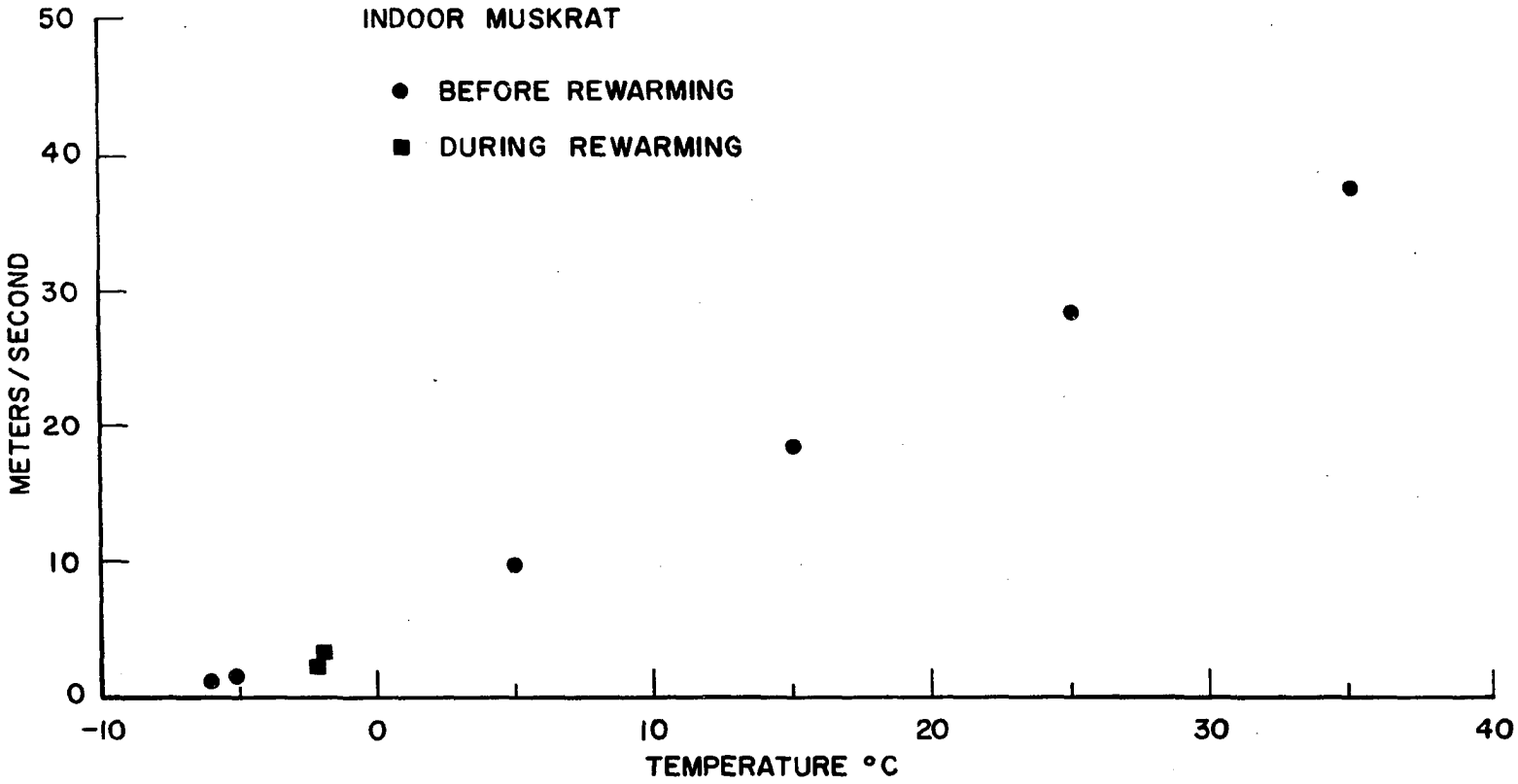

Fig. 22b. Caudal nerve conduction velocity before and during spontaneous rewarming; indoor muskrat.

rapidly by introducing Locke's solution (at room temperature) into the chamber shortly after the chamber temperature rose above 0°C. Some of the nerves for which this was not done are listed, with their temperatures of action potential reappearance, in Table 30.

In experiments in which artificial rewarming was initiated immediately after action potential extinction and in which freezing did not occur, the action potentials returned soon after the nerve temperature began to rise.

TABLE 30. Some Temperatures of Action Potential Reappearance During Rewarming Following Cooling.

Group	Extinction Temperature (°C)	Reappearance Temperature (°C)
1963 Alaska Muskrat	-4.0°	0.5°*
" "	-2.5°	0.5°
" "	-3.0°	3.0°
" "	-2.5°	>1.5°
" "	-2.5°	12°
" "	-2.5°	10°
" "	-2.5°	7°
" "	-2.0°	3.0°
" "	-3.5°	3.5°
1963 Louisiana Muskrat	-2.5°	7.5°
" "	-2.9°]	7°
" "	-3.0°	9°
" "	-2.8°	6°
" "	-2.5°	0.5°
" "	-3.0°	6°
" "	-1.2°	1°
Beaver	-5.5°	5°

* Distal recording electrode action potential reappeared at 25°C.

GENERAL DISCUSSION AND CONCLUSIONS

Past studies of temperature related mammalian nerve function have consistently emphasized the inability of peripheral nerve to conduct at the low temperatures, near 0°C or below, at which most poikilotherm nerves remain functional. The finding that caudal nerves from five of eight species of northern mammals would conduct well into the region of supercooling provides good evidence that past conclusions have been based on an inadequate sampling of mammalian fauna. This conclusion is further supported by the fact that the majority of previous studies dealing with mammalian nerve function have utilized either domestic species, such as the dog and cat, or specialized laboratory forms, such as the rat.

Most mammalian nerve studies have been further restricted to the use of internal nerves, such as the vagus, or to the sciatic and its branches. Assuming that the temperature of the immediate nerve environment can affect the thermal optimum or operating range of the nerve, temperature studies of the nerves mentioned, particularly in domestic and laboratory animals, would not be expected to demonstrate low temperature functional capability. The finding of low temperature conduction in a number of caudal and, to a lesser extent, tibial nerves in the

studies here reported may thus be attributed to two factors: First, that the animals studied come from wild populations exposed to relatively large temperature extremes; and second, that some of the nerves, i.e., especially caudal nerves, innervate body regions that undergo marked changes in temperature. That the whole matter of thermal optima in peripheral nerve may be related to more than environmental temperature is evidenced by the fact that caudal nerves of muskrats from southern Louisiana were also able to conduct at supercooling temperatures.

The relationship between conduction velocity and temperature in caudal nerves of the diffeeent species studied fits a pattern with respect to the known or presumed thermal environment of the nerve. Figure 10 demonstrated that higher conduction velocity versus temperature slopes occur in animals with well insulated or more massive tails, such as the red fox, coyote, and porcupine. The higher slopes found for caudal nerves in the better insulated animals result from either higher inherent conduction velocities (red fox and coyote) or from higher activity extinction temperatures. The latter effect is shown most clearly by the conduction velocity curve for the porcupine. The 35°C conduction velocity is about the same as the average conduction velocity at 35°C for thenerves of

those species that conduct into the supercooling region; however, the higher extinction temperature for the porcupine nerve is indicated by the greater slope of the conduction velocity versus temperature curve.

Another indication of a relationship between conduction velocity at different temperatures and the thermal environment of the nerve is provided by a comparison of caudal, tibial, and phrenic nerves. Comparing these nerves in the beaver (Figure 13), it is seen that the phrenic, accustomed to an even, internal body temperature, conducts more rapidly than the other two nerve types at 35°C. Its conduction velocity versus temperature slope is also the steepest and results in a zero conduction velocity-temperature intersection of about 3°C, or just slightly lower than the average measured action potential extinction temperature (4.5°C). The caudal nerve and tibial nerve conduction velocity slopes are similar, although the caudal nerve extinction temperatures are consistently lower. The exact thermal environments of the caudal and, to a greater extent, the tibial are not completely known, but it is certain that the unfurred tail and legs of the beaver allow significant cooling of the underlying tissue, including nerve. It is probable that the caudal nerve frequently operates in the living beaver

at temperatures between about 5° and 20°C, and the normal operating temperature for tibial nerve is probably similar. It might be expected that the closer proximity of the tibial nerve to the rather heavily muscled and fur covered upper leg might result in a somewhat warmer tissue environment, especially when the beaver is out of the water.

Further evidence for the effect of temperature environment upon nerve function is seen in the comparison between Alaska and Louisiana muskrats. The conduction velocity versus temperature curve for the warm climate Louisiana animals is steeper than the curve for the Alaska muskrats. A similar relationship, i.e., cold accustomed animals showing lower conduction velocities than warm accustomed animals at higher (25° to 35°C) test temperatures, has been reported for rats (Miller and Irving, 1963). It thus seems that exposure to low temperature results in a lowering of the action potential extinction temperature, while exposure to higher temperatures leads to increased conduction capability at higher temperatures. In the case of the Louisiana muskrats the latter effect was present even though low temperature conduction was little, if any, different from the cold climate Alaska muskrats. The finding that caudal nerves of Louisiana muskrats could conduct at supercooling temperatures was

unexpected and, therefore, of even greater interest. The detailed temperature characteristics of the Southern Louisiana muskrat habitat were not obtained, but general climatic information leaves no doubt that the Louisiana muskrats are never subjected to freezing air or water temperatures. However, water temperatures of 10° to 15°C are undoubtedly encountered in winter, and this degree of cooling may be enough to provide for the low temperature characteristics of the caudal nerves.

The other possible explanation for low temperature nerve function in the southern muskrats is that such functional capability developed during the evolution of the species, perhaps during a period of prolonged cold climate, and is a genotypic characteristic.

Alaska muskrats maintained indoors for considerable periods did not show any marked loss of low temperature nerve function. The only indication of functional deficiency at low temperatures was a slight decrease in conduction velocity, but this was not sufficient to cause any change in the extinction temperature for nerve activity. Conclusive evidence for or against the development of low temperature nerve function in the muskrat or any of the animals studied could only be obtained by keeping animals in a warm environment from the time of birth. If

the warm acclimated individuals still showed no differences in temperature related nerve activity compared with a typical wild, cold exposed population, the ability of the nerves to function in the cold would best be ascribed to heritable characteristics.

Previous thermal history had no consistent influence on nerve electrical threshold. Threshold was significantly higher in caudal nerves of Louisiana muskrats tested at lower temperatures (15°C or below) and at stimulus durations of .17 milliseconds or less. Caudal nerves of warm tailed animals such as the red fox, when compared with cold tailed species such as muskrats, failed to show such a relationship. In general the excitability thresholds determined for any given type of nerve showed considerable variation, and the alteration of threshold with temperature showed much variability in comparing individual animals within a species. It does seem surprising that the membrane or other changes associated with altered nerve function with respect to temperature are not manifested in altered excitability characteristics.

The finding by several earlier investigators (Lapicque, 1907, and Schriever, 1932) that frog sciatic nerves show an increased excitability at lower temperatures when stimuli of long duration are used is not confirmed for mammalian nerves used in the present studies.

Data pertaining to absolute refractory periods at different temperatures also fails to show consistent correlation with thermal history in caudal nerves, but a comparison of caudal, tibial, and phrenic nerves demonstrates that nerves accustomed to warmer temperatures undergo a more rapid increase in absolute refractory period as test temperature decreases, i.e., such nerves exhibit higher Q_{10} values for absolute refractory period at lower temperatures. The three types of nerve form a series with the phrenic showing the greatest increase in refractory period with decreasing temperature, tibial nerve showing a smaller coefficient of increase and caudal nerve showing the least.

Thermal history of the various nerves was correlated with the effect of temperature on action potential duration. As would be expected from action potential extinction temperatures, nerves accustomed to warmer mean temperatures showed a more rapid increase in action potential durations with decreasing temperature. Again, the phrenic showed the greatest response to decreased temperature, the caudal nerves of cold tailed species showed the least response, and tibial nerves were intermediate.

Since refractory period is related to action potential duration, it is not surprising to find similar

patterns of response to these two characteristics when comparing the three types of nerve studied. The maximum impulse frequency calculated for the different types of nerve demonstrates that the nerves that may have to function at low temperature retain the ability to conduct impulses at a rate high enough to allow normal transmission of most messages. Iggo (in Hensel, 1963) states that the maximum impulse rate for myelinated or unmyelinated thermoreceptors is about 150/second. Such conduction rates would be expected to occur in an optimum temperature region between 20° and 30°C. At temperatures below this optimum the maximal impulse frequency for a given temperature change declines. This means that an available maximum impulse frequency of 50 impulses/second, as estimated for beaver caudal nerve, should allow normal transmission of temperature and other information.

The finding that many nerves are able to conduct when supercooled provides some interesting opportunities to study the effects of freezing on nerves in the functional state and has some useful implications for previously discovered low temperature biological phenomena.

That the temperature rise associated with onset of freezing occurred within the nerve is shown by the large increases in height of the action potential and in

the conduction velocity that occurred at the onset of rewarming (Figures 19 and 21). The studies of Mazur (1963) on the thermodynamics of intracellular freezing predict that at low cooling rates, such as those used in the present studies, intracellular freezing would be unlikely at tissue temperatures warmer than -10° to -5°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 time. Nerves that were touched immediately after the disappearance of the action potential were stiff and opaque.

If intracellular ice did not form, nerve damage would be absent or minimal. The recovery of partially frozen nerves is best attributed to the lack of intracellular ice formation. Failure of nerves to recover function following freezing at -4° to -7°C fits in well with the findings of Smith (1961) and Popovic and Popovic (1963), who reported deaths in this temperature region from freezing whole mammals. The results with supercooled nerves also agree well with Moran's (1929) findings that muscle frozen below -3.5°C for 20 minutes loses its irritability on

thawing. More recently Luyet and Gonzalez (1964) found that whole muscle in rats can survive freezing for 15 minutes at -5°C and not at all at -10°C.

The need for any mammalian nerve tissue to survive freezing is a matter of conjecture, but the knowledge that nerve tissue can survive supercooling or partial freezing helps explain several well documented low temperature biological phenomena. Scholander et al. (1957) noted that fish living in the bottom of Hebron Fjord in Northern Labrador live nicely in a constantly supercooled condition at -1.8°C. Since the fish function in a coordinated manner, normal nerve activity must be present. A variety of insects and other aquatic and terrestrial invertebrates are known to survive subfreezing temperatures (Scholander et al. 1953); and although the nervous systems of such organisms are much less complex than those of mammals, basic nerve function is apparently very similar.

Evidence for recovery from freezing of peripheral nerves in man was provided recently by the observation of Mills (1965) that frozen limbs may show a return of cutaneous sensitivity after being thawed by rapid rewarming techniques. The present findings would corroborate such recoveries if nerve temperatures did not fall below -4° to -7°C.

Due to the present uncertainties and the complexities associated with the basic mechanisms of nerve transmission, it is reasonable to speculate but briefly on the changes underlying differences in nerve function at various temperatures. Such changes must manifest themselves at the membrane level where activity originates. Several studies have sought to measure the effect of temperature on ionic permeability. Frankenhaeuser and Moore (1963) utilized voltage clamp techniques to measure the rate constants and permeability constants of sodium and potassium. He found that in single toad nerve fibers the resting potential and action potential amplitude changed only one to two millivolts with a drop in temperature from 20° to 2.5°C. The Q_{10} values for sodium and potassium permeability within this temperature range were 1.3 and 1.2, respectively.

Ishiko and Loewenstein (1961) noted the differences between sense organ generator potentials and sense organ nerve action potentials with respect to temperature effects in the cat. Their observations of single nodes of Ranvier in fibers innervating Pacinian corpuscles agree well with Frankenhaeuser's findings. Electrical threshold of the nodal membrane was nearly constant between 40° and 12°C, but below 12°C the threshold increased rapidly until complete failure occurred at 8°C.

Bernhard and Granit (1946) have considered nerve as a model temperature end organ. Increased negativity of a cooled area with respect to the remaining nerve was found in cat sciatic. Such localized temperature potentials preceded actual discharge of the nerve if cooling were rapid. It might be postulated that a progressive membrane depolarization due to cooling could lead to a type of cathodal block in which case action potentials could no longer be developed. All of the permeability and electrical changes that occur at the membrane level must result from either changes in the mechanical properties of the membrane or from changes in the rate of chemical processes associated with membrane potentials. In either case nerves that are able to conduct at low temperatures may be expected to show some alteration in chemical makeup, but such alterations are yet to be determined.

As a final note on the above topic, Bernardis (1952) has demonstrated that guinea pig peripheral nerve maintains its capacity for synthesis of acetylcholine even when supercooled. If Nachmansohn's (1961) concept of the role of acetylcholine in nerve conduction is correct, acetylcholine would not appear to be the limiting factor in the ability of a nerve to function at low temperature.

Whatever the basic mechanisms it is apparent from the studies reported here that mammalian nerve is capable of activity and survival over a considerably wider temperature range than has heretofore been accepted. Taken collectively, the differences in conduction velocity, refractory period, and action potential extinction temperature in nerves accustomed to different operating temperatures offer good evidence for temperature adaptations in mammalian peripheral nerve.

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