A Comparative Study of the Effect of Temperature on Crustacean Motor Axons. (30221)

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Homarus americanus, the Maine lobster, survives well in sea water at temperatures ranging between 10° and 20°C. Survival time is limited, however, if the water temperature is raised to 26°C or cooled to 3°C. At these temperature extremes the simple reflex responses disappear, and the animal becomes quiescent. The effects are irreversible in a very short period of time. The spiny rock lobster, *Panulirus interruptus,* an inhabitant of southern waters, is quite at home in sea water at temperatures of 35° or even higher. Several specimens have been maintained in well aerated sea water at 36° for several days in the laboratory aquarium. Moreover, a number have survived for weeks at temperatures as low as 10-12°. The lower limit, lethal range, for this southern species is about 7° and the upper limit about 38°C. When subjected to these temperature "extremes," *Panulirus* exhibits similar reactions to those of the northern variety.

The disappearance of simple reflex responses in the limiting temperature ranges suggests that either nervous transmission is blocked or muscle contraction impaired, or both. If so, there is no question that these neuromuscular effects take place with warming at a far different (lower) temperature for *Homarus* than for *Panulirus.* To determine the cause of this temperature tolerance difference a comparative study of the effect of temperature on the single motor axons of both *Homarus* and *Panulirus* has been started. The first data obtained are described here and reveal a very definite species temperature sensitivity difference in the warming range.

Methods. Motor axons were isolated from the meropodite segment of the walking limb of both *Homarus* and *Panulirus* and mounted in pairs, one from each species, across 4 platinumized-platinum electrodes (100-150 μ thickness) in *Homarus* physiological solution adjusted to pH 7.6(7). Both motor axons were in contact with the same distal stimulating electrode, the anode, and the common cathode electrode which was connected with ground through a small resistor. The 2 axons were connected separately to 2 distal recording electrodes each leading to a separate beam of the oscilloscope. This method of mounting single nerve fibers across common stimulating leads and separate recording electrodes is illustrated in the inset in the lower right-hand portion of Fig. 1. Thus, the single motor nerve fiber from each lobster species was subjected to similar physiological conditions and the same stimulating current, but the response from each fiber was recorded separately. The fiber pairs were raised into oil floating on the solution and a 200-300 μ length at the cathodal region allowed to dip into this physiological solution at the oil-water interface. The solution and oil were contained in a small plastic chamber surrounded by a moat which could be flooded with concentrated saline solution of any desired temperature. For extremely low temperature studies, dry ice was placed in the moat(6). The mercury bead of a very small thermometer (Taylor special range) was placed within one mm of the fiber portion dipping into the solution at the middle cathode. It was assumed that the fiber temperature was within 0.5°C of the thermometer reading since the temperature of the entire oil-solution volume varied less than one degree anywhere in the chamber. A total of 19 paired fiber experiments have been run, including 6 medial giant fiber pairs, 8 opener fiber pairs, 3 fast-closer fiber pairs, and 2 bender fiber pairs. *Homarus americanus* specimens were obtained in good condition from Salt Water Farm, Damariscotta, Maine, where the normal environmental water temperature ranged between 15 and 18°C. *Panulirus* was taken from the ocean near Ft. Lauderdale, Fla., where the water temperature was about 25°C.

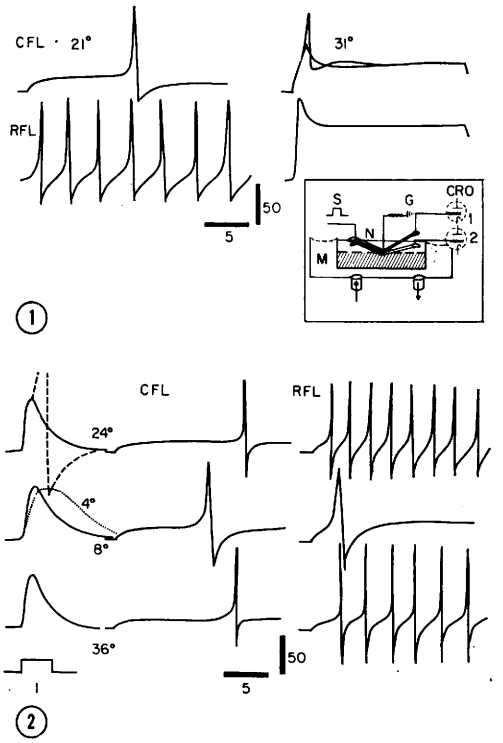

FIG. 1. Typical action potential responses recorded from *Homarus* opener axon at 21° left, and 31° right. Upper traces show response at critical firing level, CFL = 30 mv, at 21°, 100 mv at 31° with only a small spurious spike elicitable at 31°. Lower traces show oscillatory response at repetitive firing level, RFL = 35 mv at 21° on left, and only the delayed rectification response at 31°, no spikes at 120 mv stimulus. A sketch of the nerve chamber shown in lower right inset. S, stimulator pulse; G, ground; CRO, oscilloscope beams 1 and 2; M, moat; N, two nerve fibers. Physiological solution has slanted lines, O, oil, Calibration 5 ms horizontal, 50 mv vertical.

FIG. 2. Responses of a *Panulirus* opener axon at 24° upper traces, 8° middle traces (4° dashed line) and 36° lower traces. On the left are traces of the deflection caused by a subthreshold or threshold (upper traces) short duration (0.2 ms) pulse. Traces obtained at critical firing level, about 30 mv at all temperatures. Note long, 18 ms, response time at 36°. Also repetitive firing, RFL, at 36°. Calibration 1 ms on left horizontal, 5 ms and 50 mv vertical, on right.

Results. Typical data from one motor axon pair, a highly repetitive firing opener fiber pair, 88 μ and 92 μ in diameter are compiled in Table I. The Table includes critical firing (CF) levels, chronaxie, action potential voltage and duration, response time and decay rate values(6) and relative oscillatory activity. Typical responses from the *Homa-*

TABLE I. Comparative Response Data Obtained at Different Temperatures from an Opener Motor Axon of the Walking Limb of *Homarus* (H) and *Panulirus* (P) Lobsters.

	Temp °C	Critical firing level (millivolts)		Chronaxie (millisec)		Action potential				Decay rate "a" $(ms)^{-1}$		Response time (ms)		Relative repetitive firing	
						Voltage (mv)		Duration (ms)							
		H	P	H	P	H	P	H	P	H	P	H	P	H	P
	22	60	48	2.0	1.6	105	110	1	.9	2.5	2.4	15	15	++++	++++
					2.2(12°)										
Cooling	8	45	38	3.0	1.2	80	100	1.3	1.2	1.1	1.8	18	18	++	0
	5	—	38	3.0	.8	70	98	1.5	1.5	—	1.4	22	20	0	—
	22	47	52	2.2	1.2	110	100	1.1	.9	2.3	2.0	20	15	++++	—
	22	55	35	1.6	1.5	110	100	1	.8	2.4	2.5	15	18	++++	++++
	30	180	63	.25	1.35	70	100	.5	.8	3.2	2.5–2.6	3–4	18	0	++++
Warming	35	—	110	—	—	—	102	—	.6	—	2.7	—	14	—	++++
	38–40	—	150	—	.3	—	95	—	.5	—	2.9–3.0	—	10	—	++
	22	160	120	.25	.5	65	100	—	.8	2.5	2.4	4–5	14	0	++

++++ Highly repetitive. + Repetitive. 0 Non repetitive.

rus axon at 21° and 31° are presented in Fig. 1, and of the *Panulirus* axon at 24°, 8°, and 36° in Fig. 2. For a detailed study of the *Homarus* axon response at low temperatures the reader is referred to(6).

There was no significant difference between *Homarus* fiber data (H) and *Panulirus* fiber data (P) with cooling, although in the Table it might appear that the chronaxie values, the strength-duration curves, of the 2 fibers were significantly different. The all or none response of 9 out of 19 *Panulirus* axons including all medial giants and fast closers became graded at 5-6°C. The other 10 more highly oscillatory axons gave rise to small broad spikes (spurious spikes?) at 5° which became graded at 2-4°C. It was difficult, therefore, to obtain accurate and reliable excitability data in the range of 5-8°C, but at this low temperature with extinction of the all or none response the chronaxie value of the *Panulirus* fiber was markedly decreased. The chronaxie value at 12° is included for the *Panulirus* axon to show that the chronaxie actually increased as the temperature was lowered and thus simulated the change in the *Homarus* axon chronaxie value. The response of the *Homarus* axon with cooling was very little different from the *Panulirus* axon response except that the all or none spikes became graded at 2-4° instead of 5-6°, with the exception of 3, two medial giants and one fast closer (possibly in poor condition) which produced graded responses at 5° also. These low temperature data are in good agreement with previous results(6).

At high temperatures, on the other hand, there was a very significant species difference. *All* Homarus *axons were totally and irreversibly blocked at 31.5°*, except one opener fiber which responded at 35° for less than 3 minutes. Four of the 6 medial giant axons failed to give rise to a spike at 29.5°. The other 2 blocked at 31°. *Repetitive firing disappeared in all* Homarus *axons at 28-29°* with one exception of the one opener axon already cited which responded repetitively for a few seconds at 32°. The typical response of a *Homarus* axon is illustrated in Fig. 1, and shows, right-hand traces, the spurious or delayed rectification response recorded at 31°.

In striking contrast, the Panulirus *axon response, single or repetitive, was* unimpaired by temperatures elevated to 35-36°. Repetitive firing was elicitable at 38° in some instances and typical recordings are shown in Fig. 2. The *Panulirus* axon response at low temperatures, 4-8°, is the same as the *Homarus* axon response in this low temperature range(6). The *Panulirus* axon response at 24° more closely resembles the *Homarus* axon response at 18-20° in that it is extremely oscillatory. The *Homarus* axon becomes less oscillatory at 24°. When the temperature is raised to 40°, or slightly less, then the *Panulirus* axon response shows rapid inactivation and resembles the *Homarus* axon response at 31-32°C.

The membrane depolarization initiated by an applied short duration subthreshold pulse declines exponentially following the pulse, as shown in the left-hand column, Fig. 2, and described in(6). The membrane depolarization value may be approximated at any time, t, by the simple expression

$E = E_o e^{-at}$ where E_o = membrane depolarization in millivolts at time $t = 0$

a = rate constant for exponential decay of the membrane depolarization

This rate constant value which is evaluated in 1/ms or $(\text{milliseconds})^{-1}$ (Table I, Column 6), is inversely proportional to the membrane time constant ($R_m C_m$) and assuming C_m to remain constant it is possible to approximate changes for the membrane resistance values by the changes in "a" values. Using exponential curves best fitting the actual recorded decay curves for subthreshold responses at different temperatures (Fig. 2, left-hand traces) an estimate has been made in R_m with temperature for both species.

At 30° the transmembrane resistance of *Homarus* axons was reduced at least 25% and often as much as 40%, indicating a

marked permeability increase. The actual membrane resistance of crustacean axons is of the order of 1500 ohms cm^2 for opener axons, 1000 ohms cm^2 for fast closer axons, and 800 ohms cm^2 for nonrepetitive medial giant axons(5) and a 25-40% reduction would place the R_m values in the range below 1000 ohms cm^2 where oscillatory activity is lost, critical firing level significantly increased, and conduction block may occur. In marked contrast, there was no such indication of a permeability increase in most *Panulirus* fibers even at 35°C or slightly higher (Fig. 2). Indeed, there was very little change whatsoever in *Panulirus* axon characteristics unless the temperature was raised to 40°C.

Discussion. These preliminary data indicate a very definite temperature tolerance difference between nervous tissues of *Homarus* and of *Panulirus* when the temperature is elevated. As the temperature of the surrounding medium is raised to 30°C and higher the nerve fiber membrane of *Homarus* becomes (non-selectively) permeable at a significantly lower temperature than does *Panulirus* nerve fiber membranes. The reasons for this species difference are not known. There is considerable evidence that a loss of selective permeability takes place with elevated temperatures(1,2,3,4) and a pronounced permeability decrease, even selective permeability decrease, with cooling(1,2,3,6). Thus, warming produces an intra-axonal loss of potassium and gain in sodium, accounting for inactivation at certain temperatures. Cooling, on the other hand, decreases the loss of potassium and gain in sodium even with continuous stimulation(3). But, with regard to function failure with heating, the important question, "Why should the nerve fiber membrane of one species become inactivated presumably by a profound permeability shift at a significantly different temperature value (lower) than the nerve fiber membrane from another closely related species?" remains unanswered. In this regard an investigation of the effects of temperature variation on nerve fibers of a single species, such as *Callinectes sapidus,* which may be taken from both northern and southern waters, should be of considerable interest and is presently under way.

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Relationship of Age and Sex of Turkeys to Aortic Ruptures Induced by Diethylstilbestrol.* (30222)

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Treatment of male turkeys with diethylstilbestrol (DES) produces a high incidence of aortic ruptures(1). Mortality rates can be modified by manipulation of the protein, energy, and salt content of the diet(2,3). Moreover, the estrogen causes hyperlipemia and relative hypotension in treated birds. However, all of these effects were produced in males which were injected for the first time

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