Effects of Temperature on the Generator and Action Potentials of a Sense Organ

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ABSTRACT Charge transfer through the receptor membrane of the non-myelinated ending of Pacinian corpuscles is markedly affected by temperature. The rate of rise and the amplitude of the generator potential in response to a constant mechanical stimulus increase with temperature coefficients of 2.5 and 2.0 respectively. The duration of the falling phase, presumably a purely passive component, and the rise time of the generator potential are but little affected by temperature. The following interpretation is offered: Mechanical stimulation causes the conductance of the receptor membrane to increase and ions to flow along their electrochemical gradients. An energy barrier of about 16,000 cal/mole limits the conductance change. The latter increases, thus, steeply with temperature, causing both the rate of rise and the intensity of the generator current to increase.

The membrane of the adjacent Ranvier node behaves in a distinctly different manner. The amplitude of the nodal action potential is little changed over a wide range of temperature, while the durations of its rising and falling phases increase markedly. The electrical threshold of the nodal membrane is rather constant between 40 and 12°C. Below 12°C the threshold rises, and the mechanically elicited generator current fails to meet the threshold requirements of the first node. Cold block of nerve impulse initiation then ensues, although the receptor membrane still continues to produce generator potentials in response to mechanical stimulation.

INTRODUCTION

Mechanical stimulation of the receptor membrane of Pacinian corpuscles causes transfer of charges. The amount of charges transferred increases with the area of membrane excited (Loewenstein, 1959, 1961 *a*), and with the electrical gradients across it (Loewenstein and Ishiko, 1960). A plausible mechanism of the charge transfer process is that the mechanical stimulus produces an increase in conductance of the receptor membrane, and that charges move along their gradients through the membrane. This paper deals with an aspect

of the kinetics of this process, with the effect of temperature. Preliminary reports of the results have appeared elsewhere (Ishiko and Loewenstein, 1960 *a*, *b*).

METHODS

Single Pacinian corpuscles were isolated from the cat's mesentery together with a length of myelinated axon. Fig. 1 depicts the arrangement of the preparation. A copper thermode (*A*) with a gold-plated head served to change the temperature of the receptor. The thermode head was shaped to contain the corpuscle and about 10 mg of Krebs's solution covering the corpuscle. The capsule, or, in the decapsulated preparation, its nerve ending, lay in direct contact with the thermode. The heat capacity ratio of corpuscle to thermode was about 1:10,000. The myelinated axon was submerged in another pool made of lucite and filled with Krebs's solution. (In order to display details of the corpuscle, the proportions of the components of the set-up are greatly distorted in Fig. 1. It may, therefore, be helpful to give a few characteristic dimensions: Intact corpuscle, length diameter, 800 μ; transverse diameter, 600 μ; weight, 0.1 mg. Decapsulated corpuscle, length, 600 μ; transverse diameter, 8 μ. Thermode head, diameter, 2 mm. Thermode head and fins, weight, 9 gm. Water in tube *C*, weight, 10 to 20 gm.)

The temperature of the thermode was changed by flowing water of different temperatures through its fins. A thermistor of 0.5 sec. time constant, contained in the thermode head, was used to record the temperature on one of the beams of an oscilloscope. All observations on temperature effects were done at steady state levels of temperature, at last 3 minutes after the onset of a temperature change in the thermode.

In order to get an estimate of the time required for our system to attain thermal equilibrium, the following control was done. A fine thermocouple was inserted into a Pacinian corpuscle along its longitudinal axis so that it came to lie alongside the nerve ending. The corpuscle was placed on the thermode in its normal working position and subjected to steps of temperature. The temperatures of corpuscle and thermode head were simultaneously recorded. Thermal equilibrium between thermode and corpuscle was found to occur within less than 1 sec. over the entire range of temperature used in the experiments of this paper (see Fig. 1, right inset). The final temperatures of corpuscle and thermode were equal within 0.2°C, provided that the corpuscle was in contact with the thermode. Care was taken in all experiments to maintain good contact by pressing the corpuscle slightly against the thermode with the glass stylus (*S*).

The thermode served also as a recording ground lead. The electrical activity of the receptor was recorded between the region at which the axon emerges from the corpuscle and a more distant one on the axon, across a paraffin bridge one or more internodes long. The paraffin–Krebs's solution boundaries of the thermode pool and the axon were the effective recording electrodes. The electrical activity of the receptor was fed into the second beam of an oscilloscope through a condenser-coupled amplifier of 18 μsec. rise time constant and 0.9 sec. decay time constant.

Mechanical stimulation of the receptor was provided by a piezoelectrical crystal. The crystal was driven with electrical square pulses of 0.7 msec. duration and the

resulting mechanical pulses (of 0.5 to 0.7 msec. rise time constant) were applied to the receptor by means of an attached glass stylus (*S*). The mechanical pulse was monitored photoelectrically and the mechanical pulse amplitude (called *stimulus strength* hereinafter) was calibrated under a high power microscope (Loewenstein and Altamirano-Orrego, 1958).

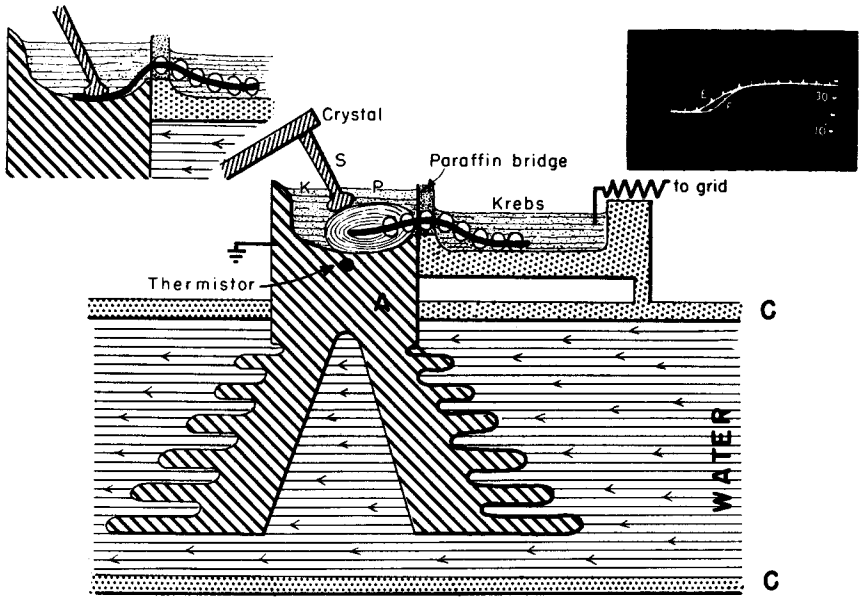

FIGURE 1. Diagram of set-up. The Pacinian corpuscle (*P*) is stimulated mechanically with the stylus (*S*) of a piezoelectric crystal, while its temperature is changed by a thermode (*A*) in direct contact with the corpuscle and recorded by a thermistor. A paraffin bridge divides two pools of Krebs's solution (*K*): one of about 0.01 cc capacity contains the intact corpuscle, its ending, and the first Ranvier node; or in some experiments, the decapsulated nerve ending (left inset); the other one, of about 0.2 cc capacity, contains a length of axon. Generator and action potentials are recorded across the paraffin bridge. (*C*), lucite tube. *Right inset*, a temperature step recorded simultaneously at the thermode (*E*, thermistor) and at the inside of a Pacinian corpuscle (*F*, thermocouple). Time calibration, 250 msec.

In a few experiments, decapsulated Pacinian corpuscles were used. The lamellae of the corpuscle were then dissected away (see Loewenstein and Rathkamp, 1958, for a description of technique), and the partially denuded ending was put in contact with the thermode (Fig. 1, left inset).

RESULTS

Temperature Effects on the Receptor Membrane

GENERATOR POTENTIAL Fig. 2 illustrates the effects of temperature on the generator potential of a Pacinian corpuscle. The corpuscle was stimulated

with a series of equal mechanical pulses and the resulting generator potentials recorded at various temperatures. The most obvious result, at high temperatures, is an increase in the rate of rise and in the amplitude of the generator potential. Both increase reversibly with temperature (8–40°C) (Fig. 3). Between 14 and 40°C, the rate of rise and the amplitude of the generator potential increase roughly linearly with temperature. In this range, the mean temperature coefficient was 2.5 for the rate of rise and 2.0 for the amplitude

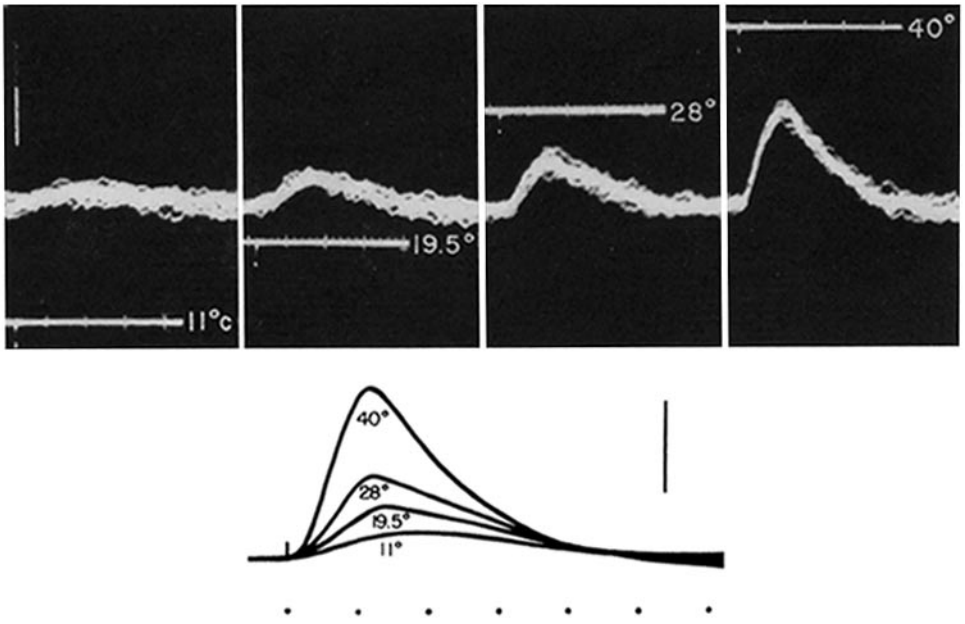

FIGURE 2. Temperature effects on generator potential. *Upper row*, the receptor is stimulated with equal mechanical stimuli and the resulting generator potentials are recorded at different temperatures. Seven successive oscilloscope sweeps taken at a frequency of 10/sec. are superimposed on each photograph. The second beam records temperature. *Lower row*, tracings of the upper records slightly enlarged. Calibration, 25 μv; 1 msec.

of the generator potentials. Table I summarizes the results of four representative cases.

The generator potential is known to be non-linearly related to mechanical stimulus strength. In the high range of strength, where the non-linearity is most pronounced, the temperature coefficient of the generator potential is found to diminish noticeably. This is illustrated by the experiment of Fig. 4 *A* and *B*, in which the receptor was stimulated with three different strengths in the ratio of 2:3:5. The ordinates of the corresponding generator potential strength curve (Fig. 4 *C*) give approximately the strength magnitude in proportion to the maximal generator potential. Over the range of strength where

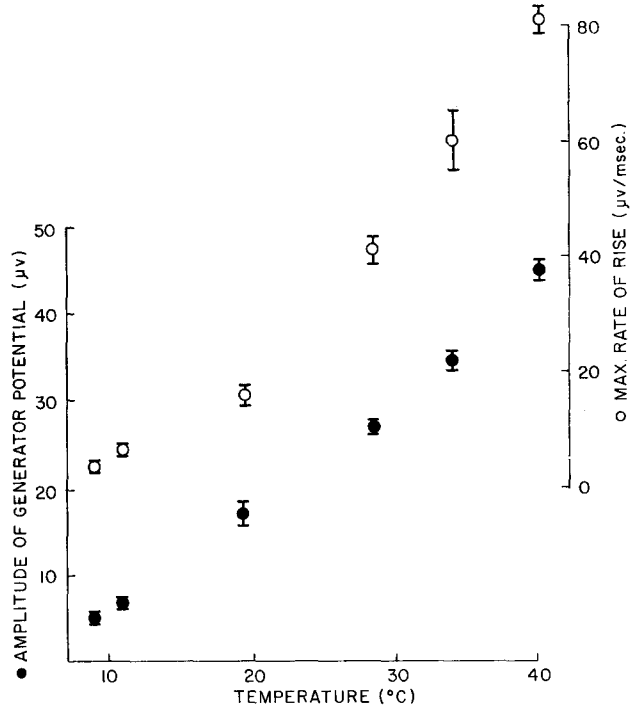

FIGURE 3. Effect of temperature on the amplitude and rate of rise of generator potential. *Abscissa*, temperature of receptor. *Ordinates*, ●, mean values of the amplitude, and ○, of the maximal rate of rise of 35 to 50 generator potentials. Bars subtend the standard error of the mean.

TABLE I

TEMPERATURE COEFFICIENTS (Q_{10}) AND ACTIVATION ENERGIES (E_a) OF GENERATOR POTENTIAL

Experiment No. and preparation	Temperature range	Amplitude (Q_{10})	Maximum rate of rise		Rise time (Q_{10})	Decay time constant (Q_{10})
			(E_a) K cal/mole	(Q_{10})		
T-2 (intact corpuscle)	13.5–32.5°C	1.9	16.4	2.4	−1.5	−1.2
T-4 (intact corpuscle)*	15–35°C	1.8	16.1	2.5	−1.1	−1.2
T-14 (intact corpuscle)		2.3	16.2	2.6	−1.1	−1.2
S-6 (decapsulated preparation)	4–33°C	2.0	16.2	2.6	−1.2	−1.2
Average		2.0	16.2	2.5	−1.2	−1.2

* Node blocked with procaine (0.5 per cent). Case represented in Figs. 2 and 3.

generator potential and strength are more linearly related, the temperature coefficient is relatively constant. This is the range in which the temperature coefficients given in Table I were obtained. But as the derivative of the gen-

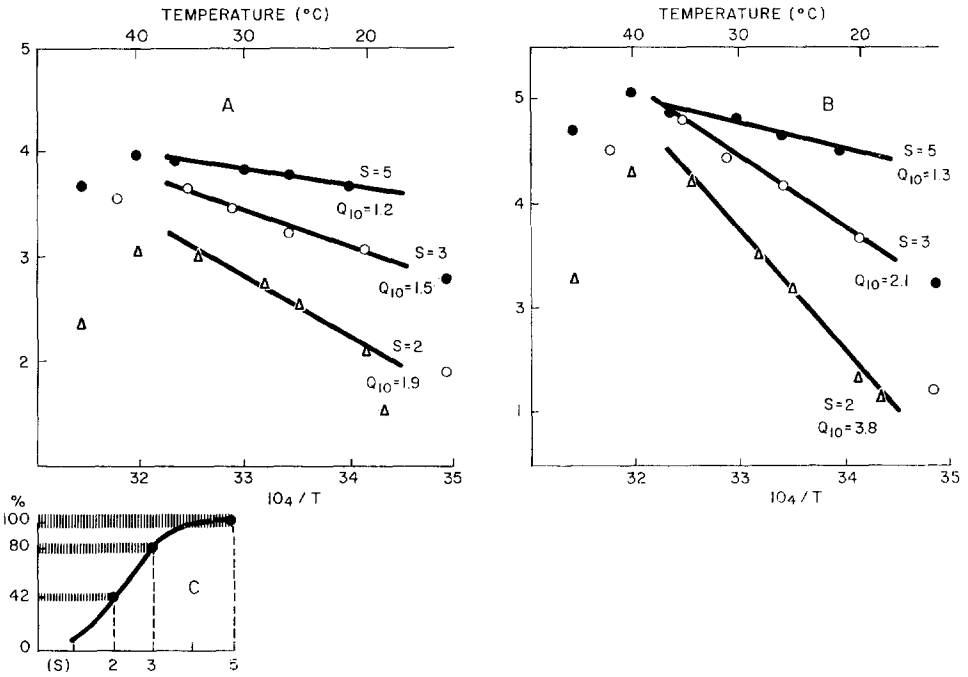

FIGURE 4. Temperature coefficient of generator potential at different stimulus strengths. The receptor is stimulated with three different strengths (S) in ratios of 2:3:5, and the temperature is varied for each strength. *Abscissae A* and *B*, reciprocal of absolute temperature. *Ordinates*, *A*, $\log_n$ of mean amplitude; *B*, $\log_n$ of mean rate of rise of generator potential. Each point is the mean of 30 to 50 cases; standard error in *A* is less than 2.7 per cent and in *B*, less than 4.4 per cent. Curves drawn by least squaring. The slopes give the activation energy; the corresponding temperature coefficients are indicated on each curve. *C*, generator potential–strength relation at 30°C. *Abscissae*, stimulus strength S; *ordinates*, fractions of maximal generator potential amplitude (specified as ranges because of unsharp definition of maximal generator potential).

erator potential–strength curve diminishes, the temperature coefficient diminishes markedly (Fig. 4).

The falling phase, presumably a purely passive component of the potential, and the rise time of the generator potential are but little affected by temperature (Fig. 5). The time constant of decay and the rise time of the generator potential have mean temperature coefficients of -1.2. These coefficients are independent of stimulus strength.

In three corpuscles, the capsule was removed and the denuded ending was

placed in contact with the thermode. Under these conditions the generator potential can be led off directly from the ending, while the latter is being subjected to varying temperature. The generator potential was then found to vary with temperature in essentially the same manner as in the intact corpuscle. The data of the last case in Table I are from a decapsulated preparation.

All observations were done at steady state levels of temperature. Thermal equilibrium is reached within less than 1 sec. in the intact corpuscle (see Methods) and even more rapidly in the decapsulated ending. To insure an

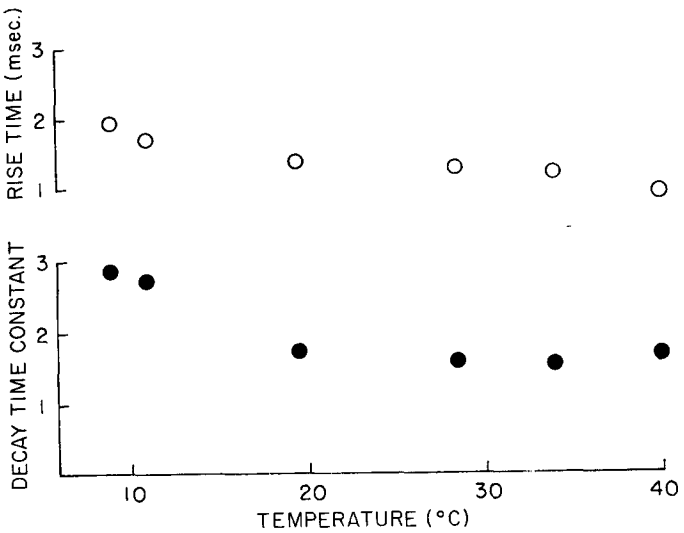

FIGURE 5. Effect of temperature on the rising phase and falling phase of generator potential. *Ordinates*, rise time (○), and decay time constant (●) of generator potential mean value of 35 to 50 generator potentials (same cases as in Fig. 3), standard error less than 5 per cent. *Abscissae*, temperature.

ample margin of safety in equilibration time, all measurements of temperature effects on generator and action potentials reported in this paper were done with a delay of at least 3 minutes after the onset of a temperature change. Moreover, another control was available: After the application of a temperature step, the receptor was stimulated with constant mechanical pulses at a given frequency. The resulting generator potentials were monitored for 20 to 30 minutes to insure that steady state conditions were prevalent.

Temperature Effects on the Nodal Membrane

ACTION POTENTIAL The present preparation provided us with the opportunity to examine side by side the effects of temperature on two distinctly different membranes: the receptor membrane of the nerve ending and the

membrane of the adjacent axon. The former, which is the site of mechano-electric conversion (Loewenstein and Rathkamp, 1958), has a finely graded electrical output. The latter, the site of nerve impulse initiation (namely, its first node of Ranvier) has an output of the all-or-nothing kind. It responds

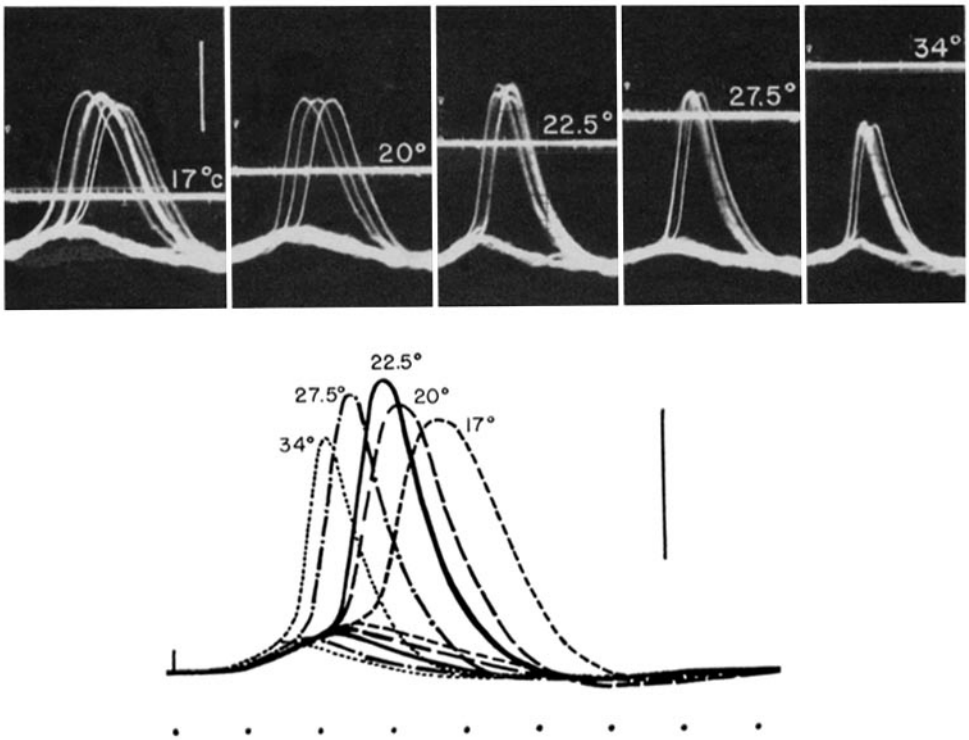

FIGURE 6. Firing of action potentials at five temperatures. The strength of the mechanical stimulus is adjusted at each temperature level to be critical at threshold for firing of action potentials. The height of the dot over the temperature beam gives the relative magnitude of stimulus strength. Seven successive oscilloscope sweeps taken at a frequency of 10/sec. are photographed on each record. Impulse conduction has been blocked at the 2nd and 3rd Ranvier nodes to obtain monophasic action potentials. *Bottom picture,* enlarged tracings of the upper records; action potentials of longest latency were traced. Calibration, 100 μv; 1 msec.

with a typical action potential to a generator current of sufficient intensity. Fig. 6 illustrates the effects of temperature on the action potential of the axon. In contrast to the generator potential of the receptor membrane, the amplitude of the action potential is little altered by temperatures between 20 and 40°C. Within this range, the amplitude has a negative temperature coefficient of 1.2 (Fig. 7). Between 20 and 12°C, the amplitude of the action potential decreases sharply, and around 12°C, action potentials are no longer elicitable.

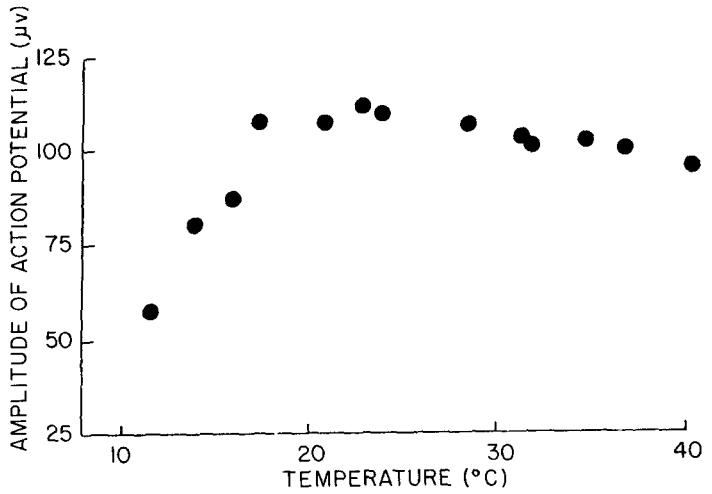

FIGURE 7. Effect of temperature on action potential.

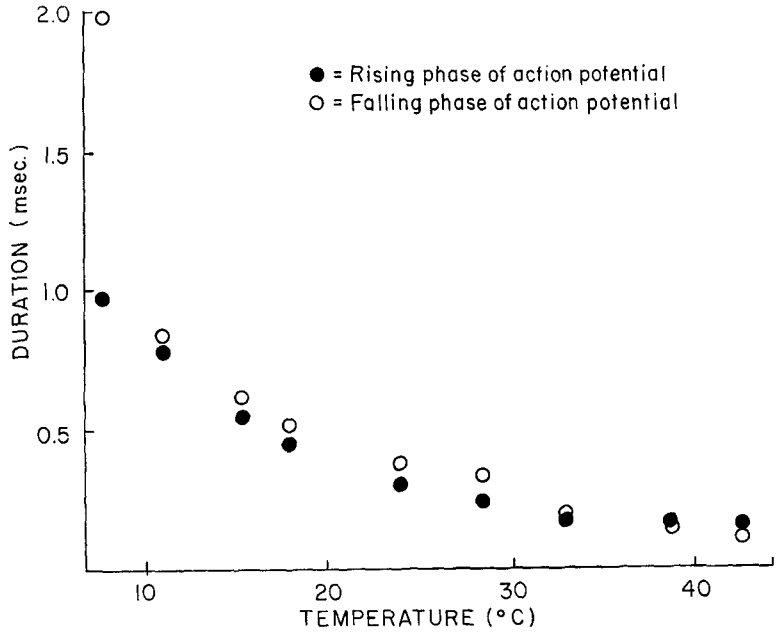

FIGURE 8. Effect of temperature on rising and falling phase of the action potential.

The duration of the action potential changes markedly with temperature. Unlike the generator potential, the duration of both the rising phase and of the falling phase of the action potential increases with a fall in temperature (Fig. 8).

Thus, in their behavior with temperature, the Ranvier nodes of the Pacin-

ian corpuscle resemble other single cell structures with all-or-none responsiveness, such as the squid giant axon (Hodgkin and Katz, 1949), frog nerve fibers (Tasaki and Fujita, 1948; Autrum and Schneider, 1950; Schneider, 1950; Hodler *et al.*, 1951), frog muscle fibers (Nastuk and Hodgkin, 1950); frog and cat heart muscle fibers (Woodbury *et al.*, 1951; Trautwein *et al.*, 1953; Coraboeuf and Weidmann, 1954), and eel electroplates (Schoffeniels, 1958). (For a comparison with other excitable tissues, see Bernstein, 1902; Gasser, 1931; Bremer and Titeca, 1934; Cardot and Arvanitaki, 1941; Auger and Fessard, 1936; Lorente de Nó, 1947; Lundberg, 1948; Tasaki and Spyropoulos, 1957; Burkhardt, 1959).

Action potentials were produced by either (*a*) mechanical stimulation of the receptor membrane that produced generator currents of just critical strength to trigger an action potential at the first node of Ranvier; or directly by (*b*) electrical stimulation of more central regions of the axon with square pulse current of 0.05 to 0.1 msec. duration and 3 μsec. rise time constant of just threshold strength. Identical temperature effects on the amplitude and duration of action potential were obtained by the two methods.

FIRING THRESHOLD An action potential is discharged at the first Ranvier node whenever the generator potential reaches a critical amplitude (henceforth called *firing threshold*). Fig. 9 *B* illustrates the effects of temperature on the firing threshold of the first node. The strength of the mechanical stimulus was adjusted at each temperature level to bring the generator potential to the critical firing threshold. As the temperature is decreased, the firing threshold increases slightly between 40 and 20°C, and steeply below 20°C. Complete failure of impulse production occurs around 12°C.

If the transducer mechanism of the receptor membrane is bypassed, and the axon is stimulated with square pulse current from an outside source, the threshold for impulse firing is found to be practically unchanged over the range of 40 to 12°C. A significant rise in threshold is seen only at temperatures below 12°C (Fig. 9 *A*). It appears, therefore, that the rise in nodal firing threshold in the range of 40 to 12°C of Fig. 9 *B* is largely due to a decrease in the rate of rise of the generator potential (see Fig. 6). Complete failure of impulse production by electrical stimulation occurs at temperatures below 8°C.

THRESHOLD FOR ADEQUATE STIMULATION The amplitude and the rate of rise of the generator current increase with area of excited receptor membrane. The area, in turn, increases with mechanical stimulus strength (Loewenstein, 1959, 1961 *a*). Thus, a simple way to vary the amplitude and the rate of rise of the generator potential is to change the strength of the mechanical stimulus (Álvarez-Buylla and de Arellano, 1953; Gray and Sato, 1953). The minimal strength of mechanical stimulus (*mechanical threshold*) applied to the receptor

membrane, required for eliciting an action potential from the first node depends on (*a*) the amplitude of the generator potential; (*b*) on the rate of rise of the generator potential; and (*c*) on the electrical threshold of the node

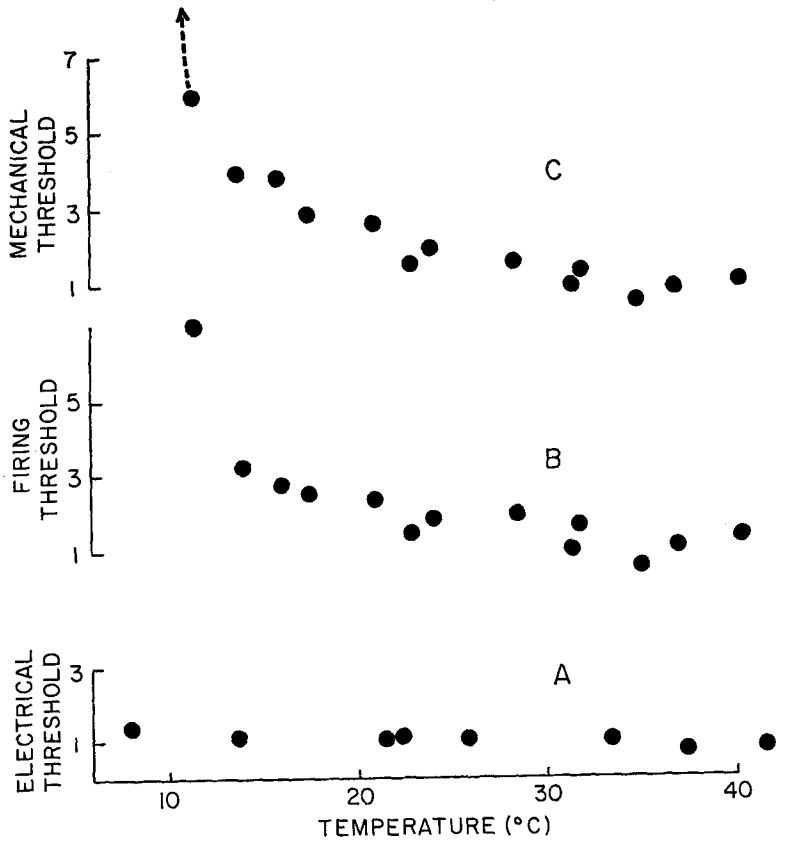

FIGURE 9. Effect of temperature on the threshold of firing of action potentials. *Abscissae*, temperature. *Ordinates*, *A*, minimal strength of current that trips off an action potential; *B*, critical amplitude of generator potential for firing of impulses; *C*, minimal strength of mechanical pulse that satisfies firing threshold *B*. In *A*, the axon of the corpuscle is stimulated with electrical square pulses. In *B* and *C*, the receptor is stimulated with mechanical pulses; data from same receptor as in Fig. 7. Standard error is less than 5 per cent in all cases.

(*cf.* Loewenstein and Ishiko, 1960). The electrical threshold of the first node is likely to remain rather constant between 40 and 12°C like that of the more central nodes (Fig. 9 *A*). In this temperature range, the mechanical threshold may be expected to depend chiefly on the amplitude and the rate of rise of the generator potential. Since both decrease with temperature (Fig. 3), the mechanical threshold will expectedly increase (Fig. 9 *C*). As the temperature

falls below 12°C, and the rise in electrical threshold enters into play, the transducer mechanism of the receptor membrane fails to keep pace with the enhanced threshold requirements of the node, presumably because the stimulus strength–generator potential relationship becomes saturated. Action potentials are then no longer elicitable at any mechanical stimulus strength. As an information device for the organism, the sense organ is then effectively "anesthetized," although at this temperature, and even a few degrees below,

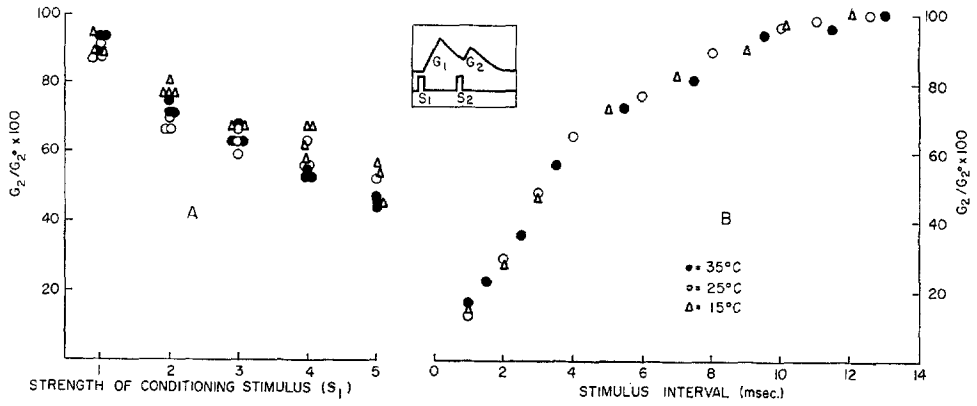

FIGURE 10. Temperature and the refractory state of the generator potential. *A*, the receptor is stimulated with two successive mechanical stimuli at constant interval (3 msec.) and constant test strength (S_2). The ratio conditioned (G_2) to unconditioned test generator potential (G_2^o) is determined as a function of conditioning stimulus strength (S_1) at 15, 25, and 35°C. Values of S are relative units of displacement amplitudes of the stimulating crystal. *B*, the receptor is stimulated at constant test and conditioning strengths ($S_1 = 5$), and G_2/G_2^o is determined as a function of stimulus interval.

the receptor membrane continues to produce generator potentials in response to mechanical stimulation and the nodal membrane to produce action potentials in response to electrical pulses, provided they are sufficiently strong and steep.

The Refractory State of the Receptor Membrane and Temperature

Excitation leaves a refractory-like condition in the receptor. If two mechanical stimuli are applied in succession so that the second stimulus falls on the refractory trail of the first, the amplitude of the second generator response is directly related to the stimulus interval and inversely to the strength of the first stimulus (Gray and Sato, 1953; Loewenstein and Altamirano, 1958). The effect of temperature on the refractory condition is examined in the following experiments.

A mechanical stimulus (S_2) of a given strength is applied to the receptor,

and the amplitude (G_2^o) of the resulting generator potential is measured. A conditioning stimulus (S_1) is then applied 3 msec. before S_2; the amplitude (G_2) of the test generator potential is thereby clearly reduced below its unconditioned value G_2^o. The ratio G_2/G_2^o is then determined at three different temperature levels for a series of S_1 values (Fig. 10 *A*). A change in temperature is found to vary G_2 merely in the same proportion as G_2^o.

The time factor of refractoriness is also independent of temperature. Fig. 10 *B* illustrates the results of an experiment in which the interval between con-

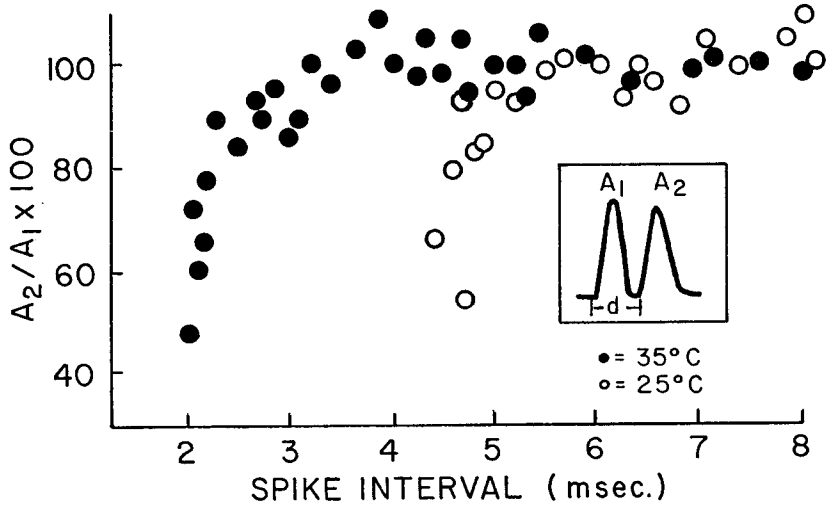

FIGURE 11. Temperature and refractory state of action potential. Recording of action potential at 25 and 35°C. *Abscissae*, interval between action potentials. *Ordinates*, ratio of test (A_2) to conditioning action potential (A_1).

ditioning and test stimuli of constant strengths is progressively increased. The time course of recovery of the test generator potential is seen to be unchanged over a wide range of temperature. This contrasts with the marked temperature dependence of the refractory period of the adjacent nodal membrane (Fig. 11).

DISCUSSION

The preceding results revealed that the mechanically elicited generator potential is strongly temperature-dependent. The question that here presents itself is whether the described results on generator potential do actually reflect temperature effects on the excitation process of the receptor membrane, or whether they reflect merely mechanical changes of extrinsic material of the sense organ. For the purpose of this discussion it may be helpful to divide our preparation into two parts: (*a*) the fluid-filled multilayered capsule of the corpuscle, and (*b*) the receptor membrane of the nerve ending with its intra-

and extracellular fluid, the latter being separated from the capsule fluid by the first lamella around the ending. The *a* represents the extrinsic elements, and the *b*, the intrinsic structural elements of the excitation process. That the observed results on generator potential are due to changes in the extrinsic elements, such as viscoelastic changes, may be ruled out: the temperature coefficient of the intact corpuscle was found to be essentially the same as that of the denuded nerve ending after removal of the capsule. Besides, the high temperature coefficient of the generator potential (2.0 to 2.5) makes such a possibility already *a priori* unlikely. The viscosity dependence on temperature of the capsule and its fluid content is not expected to differ from that of other hydrocolloids whose coefficients of viscosity are approximately proportional to the absolute temperature. For instance, the temperature coefficient of viscosity of blood plasma (Snyder, 1911) and egg albumin (Sutherland, 1908) is as low as that of water (1.2 between 20 and 40°C).

Another possibility, that of the observed temperature effects being due to changes in length constant of the passive myelinated axon, may also be dismissed. A change in length constant may be expected to work in the wrong direction for the amplitude change of generator potential. The length constant of other nerve and muscle fibers is known to diminish with increasing temperature (Hodler *et al.*, 1951; Tamashige, 1950; del Castillo and Machne, 1953; Coraboeuf and Weidmann, 1954); there is no reason to believe that the length constant of the nerve fiber of Pacinian corpuscles behaves differently, especially since the falling phase of the generator potential (the passive component of the potential) was found to have a negative temperature coefficient (-1.2). But, regardless of the direction in which the length constant changes with temperature, it cannot account simultaneously for the observed increase in amplitude and in rate of rise of the generator potential.

We may conclude, therefore, that the observed temperature effects take place at the level of the receptor membrane. What stage of the excitation process at the receptor membrane is affected cannot be said without resorting to a particular model. The only direct inference that can be made from the present results is that temperature does not simply affect the stimulus efficacy. A consideration of the generator potential (G)—stimulus strength (S) relationship at varying temperatures allows one to exclude an effect of temperature on the stimulus efficacy of the types:

(1) $G = G(aS)$, where a is some function incorporating the entire temperature dependence of G,

or,

(2) $G = G(S^n)$, where n incorporates the entire temperature dependence of G,

because the G *vs.* S curves for various temperatures cannot be made to coin-

cide by shifts along the abscissa axis, when the abscissa is plotted on either (*a*) a log scale or (*b*) a log log scale (Fig. 12).

Perhaps the simplest explanation of the observed effects on generator potential is that temperature increases the conductance in the mechanically excited receptor membrane. The entire receptor process has been shown to take place at the non-myelinated nerve ending, whose membrane appears to be the receptor membrane proper (Loewenstein and Rathkamp, 1958). Absorption of mechanical energy in this membrane leads to transfer of charges; namely, to the flow of generator current. The present results indicate that

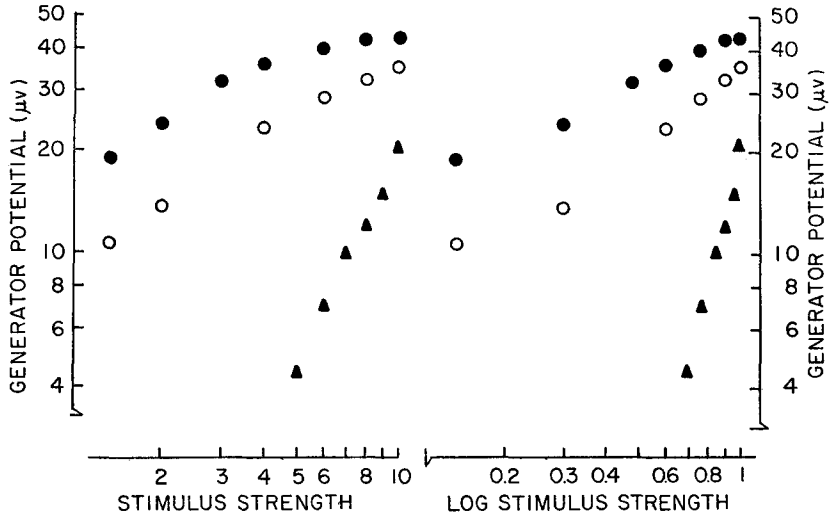

FIGURE 12. Generator potential amplitude–stimulus strength relation at three temperatures: ●, 35°C; ○, 25°C; ▲, 15°C (abscissae and ordinates on logarithmic scale).

this process (hereinafter referred to as *excitation*) has a high temperature coefficient. This may mean that there is a high potential energy barrier at some stage of excitation. The energy of activation of the hypothetical rate-limiting step in excitation may then be calculated from the temperature dependence of the rate of rise of the generator potential. It amounts to about 16,000 cal/mole in the approximately linear range of the stimulus strength–generator potential curve (Table I and Fig. 4). Previous experiments had shown that charge transfer is an increasing function of the electrical gradients across the receptor membrane (Loewenstein and Ishiko, 1960). We will propose, therefore, the following tentative scheme of excitation: *The receptor membrane separates two media of different ionic concentration. Mechanical stimulation causes the permeability of the receptor membrane to increase and ions to flow through along their electrochemical gradients. An energy barrier of about 16,000 cal/mole limits this process.* The change in permeability may be directly coupled with the mechanical stimulus; or it may be mediated through a chemical reaction which repre-

sents, then, the rate-limiting step. We will here confine the discussion to the former mechanism. The excitation scheme pictures, in this case, essentially a mechanosensitive diffusion model. The value of 16,000 cal/mole obtained in the present experiments may then seem rather high. It would be high, indeed, for diffusion in bulk solutions where the Einstein equation approximately holds, but it is not unusually high for thin surface films. In a monolayer, due to lateral association of the surface molecules with one another and solvation of polar groups, diffusion may have much higher activation energies than in the corresponding bulk solution. An example is the diffusion of water through a fatty acid monolayer, with an activation energy as high as 14,500 cal/mole (Archer and LaMer, 1955). It is interesting that the resistance to diffusion through such a monolayer decreases with decreasing surface pressure (Rosano and LaMer, 1956). This provides us with a simple mechanosensitive diffusion model which may be helpful in visualizing how an excitation process, like the one proposed above, may work: Stretching of the model monolayer decreases the lateral attractive forces between its molecules and, thereby decreases the resistance for diffusion through the monolayer. Stretching of the receptor membrane may be imagined to have a similar effect on the lateral forces between its constituent molecules, lowering the resistance for ion diffusion; or stated simply, to stretch out diffusion "pores" in the receptor membrane (see also Katz, 1950). Ions diffuse through the pores along their electrochemical gradients; the net transfer of ionic charges constitutes the generator current. It is interesting in this connection that stretch causes changes in conductance also in membranes that are not properly mechanoreceptors. For example, stretching of red blood cells (Davson, 1937), muscle fibers (Ishiko, 1958), and certain non-myelinated axons (Goldman and Julian, 1960), causes an increase in membrane conductance.

The value of 16,000 cal/mole of activation energy was obtained in the approximately linear range of the stimulus strength–generator potential curve. But, as may be seen from the example of Fig. 4, the Q_{10} still increases as the strength is decreased beyond that range. Consequently, since, we are not measuring conductance directly (and conductance is probably the primary temperature-related factor here), values closer to a true activation energy would be obtained in the lower range of strength, where the generator potential is more likely to be proportional to the conductance change (see, for example, Loewenstein, 1959, p. 384). However, it is often difficult to produce good generator potentials in this range; moreover, the shape of the strength–generator potential curve and the Q_{10} in the low strength range vary too much in different receptors to give meaningful averages. We have preferred, therefore, the linear range, where the Q_{10} is fairly constant in different receptors and rather independent of stimulus strength (Table I). The true value of activation energy is thus expected to be higher than 16,000 cal/mole, probably about 20,000 cal/mole.

We may try now to examine the observed temperature effects in the light

of the above excitation scheme. The most striking result here is that not only the rate of rise, but also in contrast to the behavior of the action potential, the amplitude of the generator potential increases with temperature (Fig. 3). A possible explanation that presents itself is that the effects are caused by an increase in resting potential. This seems, however, unlikely. In all excitable membranes heretofore examined, the resting potential was found to remain rather constant with temperature, or to have temperature coefficients near to unity (Ling and Woodbury, 1949; Hodgkin and Katz, 1949; Woodbury *et al.*, 1951; Trautwein *et al.*, 1958; Coraboeuf and Weidmann, 1954).

A more likely explanation may be given by relating temperature to the permeability change of the excited receptor membrane; that is, by assuming that the conductance change in the *excited* membrane is enhanced by increasing temperature. Several pieces of evidence suggest that the excited receptor membrane, like the acetylcholine–receptor membrane of the motor endplate (Fatt and Katz, 1951, *cf.* Grundfest, 1957), may act like a relatively *non-selective* ion sink where excitation approaches the condition equivalent to short-circuiting the receptor membrane with a leak resistor (Diamond *et al.*, 1958; Loewenstein and Ishiko, 1960; Loewenstein, 1961 *a*). We assume here that temperature increases this short-circuiting action.[1] An explanation of this sort fits the results rather well. It accounts for the fact that both the rate of rise and the amplitude of the generator potential increase with temperature (Fig. 3). It also helps one to understand why, in the case of the action potential of the adjacent axon membrane where the resting potential across the membrane shifts to a given new level during excitation by a *selective* permeability change (*cf.* Hodgkin and Huxley, 1952; Dodge and Frankenhaeuser, 1958), only the rate of rise, and not the amplitude of the potential, is sharply temperature-dependent.

In this view, the observed temperature coefficients of generator potential reflect either a rate-limiting activation energy associated with ion flow through the receptor membrane, or an activation energy associated with the alteration of the membrane structure responsible for the permeability change. It will be noted, however, that this does not imply interchangeability of mechanical and thermokinetic energy in excitation, as might, for example, be suggested by the observed inverse relationship between temperature coefficient and stimulus strength (Fig. 4). On the contrary, a complementariness of this sort is quite unlikely. In experiments designed to examine the question of thermal ex-

[1] In this respect a comparison with the temperature effects at the motor endplate would be interesting. Indeed, the amplitude of the endplate potential (but not of the miniature potential) elicited by motor nerve stimulation increases with temperature (Eccles *et al*, 1941; Boyd and Martin, 1956; Takeuchi, 1958). However, the effect may be largely on transmitter release and is thus not strictly comparable with the effects on the receptor membrane reported here. It would be desirable to get information about temperature effects on the endplate potential elicited by directly applied acetylcholine; a more valid comparison would then seem possible.

citability of the receptor membrane, no generator potentials could be elicited with temperature rise rates as high as 38°C/sec. (Loewenstein, 1961 *b*). Thus, the agent that initiates the excitation process is the mechanical stimulus; temperature appears merely to modify the process.

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