WARMING-INDUCED HYPERPOLARIZATION OF CARDIAC MUSCLE CELLS AND SNAIL NEURONES: INTERPRETATION BASED ON TEMPERATURE TRANSITION OF COOPERATIVELY LINKED SURFACE ANIONIC SITES BETWEEN K' AND Na^+ ADSORBING STATES

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• ***The resting potential of a variety of living cells exhibits pronounced hyperpolarization when the cooled cells are brought to a higher temperature. It was shown that this phenomenon can be quantitatively described by the surface adsorption theory of the cell electricalpotential in terms of a cooperative temperature transition of the protein-ion-water system at the cell surface. As such, the basic mechanism resembles the temperature transition demonstrated for the bulk phase $K^+ - Na^+$ distribution in these and similar cells. The paper also discusses the resting potentials of frog muscles and squid axons which do not exhibit hyperpolarization on warming.***

Hodgkin and Katz's ionic theory of the cellular electric potential has guided much fruitful research in cellular electrophysiology in the last three decades (Hodgkin and Katz, 1949a, Katz, 1966, Hodgkin, 1971). Conceptually the ionic theory is built on the membrane-pump theory of the living cell. The basic tenets of the membrane-pump theory include the following: (i) the bulk of cell K' exists in a free state and (ii) the cell has more than enough energy to operate ***all*** the postulated pumps. The validity of both of these tenets has now become seriously in doubt. Thus in frog muscle cells, K' is not free and evenly distributed in cell water. Rather it is adsorbed at specific sites at the A bands and Z-lines (Ling, 1984). There is not enough energy to operate the pumps in frog muscle cells. The Na pump alone has been shown to require at least 15 to 30 times as much energy as the total energy available to the cell (Ling, 1962, 1984). Three remedial postulations intended to save the Na pump theory have all been experimentally disproven (for brief review, see Ling, Walton, and Ling, 1979; Ling, 1984).

An alternative theory of the cellular electrical potential, nearly as old as the ionic theory, is the surface adsorption (SA) theory (Ling, 1955, 1960, 1962), a subsidiary part of the association-induction (AI) hypothesis (Ling, 1962, 1984). In the different versions of the membrane theory, including the ionic theory of Hodgkin and Katz, the resting potential ($) depends on the permeabilities of the membranes to ions. In the SA theory, ψ depends on the density and nature of fixed ionic sites at the cell surface, the concentrations and nature of counterions of the opposite charge in the surrounding medium, the affinities of the fixed ions for each of these counterions, and the absolute temperature. In normal resting frog muscle, the fixed ionic sites are the isolated β- and γ-carboxyl groups with preferential affinity for K' over Na' and no affinity for divalent cations (e.g., Mg^{++}) or anions (e.g., Cl^-). These and other characteristics enable the SA theory to ex-

plain experimental facts that support the Hodgkin-Katz theory as well as those that do not support it (Ling, 1983, 1984).

Bernstein's original membrane theory (Bernstein, 1902, 1912), and Hodgkin-Katz's ionic theory, as well as the SA theory, all explicitly describe a quantitative dependence of the resting potential (ψ) on absolute temperature (T). Within the limitation of the technique available, Bernstein was the first to report confirmation of the dependence of his measured "injury potential" on the absolute temperature (Bernstein, 1902). With the capillary glass electrodes, Ling and Woodbury (1949) were able to obtain more precise confirmation of the dependence of the resting potential of frog sartorius muscle on the absolute temperature. Hodgkin and Katz (1949b) showed that between 0° to 20°C, the resting potential remained constant, and in 1954 Corabeuf and Weideman reported constancy of the resting potential of Purkinje fibers. of calf and sheep hearts between 25" and 40°C, both suggesting a weak dependence of the potential on absolute temperature. However, it should be mentioned that the resting potential of frog sartorius muscle, squid axon, and mammalian heart muscle all declined at temperatures beyond the reported upper limits of constant ψ, which were 30°C for frog muscle, 20°C for squid axon, and 40"C for sheep and calf Purkinje cells. With good reasons, the investigators believed that these high temperatures brought about irreversible and unphysiological changes. This reason was probably also why the substantial decline of ψ with cooling below 20°C seen in the heart muscle of calves and sheep (Corabeuf and Weideman, 1954) was not emphasized, since the tissues were from warm-blooded animals whose physiologically compatible conditions include a narrow range of warm temperature. However, in retrospect, the observed decrease of ψ at low temperature might not have been irreversible and thus might have greater significance than realized at the time (see below).

Kerkut and Ridge (1961) measured at different temperatures the resting potentials of muscle fibers from three animals: crab (Carcinus naenas), cockroach (Periplaseta americana), and frog (Rana temporaria). At moderate temperatures above freezing, a physiological parameter that varies with the absolute temperature has a Q_{10} from 1.034 (303°/293°) to 1.037 (283°/273°). Kerkut and Ridge showed that the Q_{10}'s of the ψ's of crab muscle and frog muscle were 1.06 and thus close to the results of Ling and Woodbury. In contrast, the Q_{10} for cockroach muscle was much larger, close to 1.3. These studies seemed to indicate species-dependent differences of the Q_{10} of ψ. Some showed ψ's that vary with absolute temperature; others did not. Snail neurones, however, showed a Q_{10} that varied between 1.04 and 1.96 (Kerkut and Ridge, 1962). Within the next decade, the high Q_{10}'s of the resting potential observed by Kerkut and Ridge in cockroach were observed in a large variety of other living cells, including molluscan neurones (Kerkut and Ridge, 1962; Carpenter and Alving, 1968; Gorman and Marmor, 1970); crustacean muscle (Senft, 1967) and mammalian tissues including voluntary muscle (Akaike, 1975); smooth muscle of rabbit myometrium (Kao and Nishiyama, 1969); rat myometrium (Taylor et al., 1970); guinea pig taenia coli (Tomita and Yamamoto, 1971); and heart muscles (Page and Storm, 1965; Tamai and Kagiyama, 1968; McDonald and McLeod, 1971).

To this date, virtually all interpretations offered for these observations are based on the membrane-pump theory. Insensitivity of ψ to temperature, as in squid axon, is seen as an example following strictly the ionic theory of Hodgkin and Katz. Greater sensitivity of ψ to temperature is seen as a composite of two types of potentials: one is ionic, following the Hodgkin-Katz equation (in its modified form); the other is "metabolic" due to an

altogether different mechanism (an electrogenic pump) (Gorman and Marmor, 1970).

In view of the strong evidence against the membrane pump theory, partly mentioned above, and other evidence described elsewhere (see Ling, 1984) it seems desirable to seek alternative interpretations of resting potentials. The purpose of this report is to present a different interpretation of the temperature dependence of the cellular resting potential, based on the surface adsorption theory.

Temperature Transition of K^+ and Na^+ Distribution in Whole Mammalian Cells. Tissues of various homeotherms, including chicken embryo muscle (Brues et al., 1946); canine carotid arteries (Jones, 1973); rabbit myometrium (Jones, 1970); guinea pig taenia coli (Reisin and Gulati, 1973); and human lymphocytes (Negendank and Shaller, 1980) undergo a reversible loss of K and gain of Na^+ on cooling. As a rule, the observed data quantitatively follow the cooperative adsorption isotherm of Yang and Ling (Ling, 1964) or more precisely, the general equation for solute distribution given by Ling (1965) which includes the Yang-Ling isotherm as its major component (the second term on the right of Equations 1 and 2 below). For K and Na^+ distribution, the equations appear in the following forms:

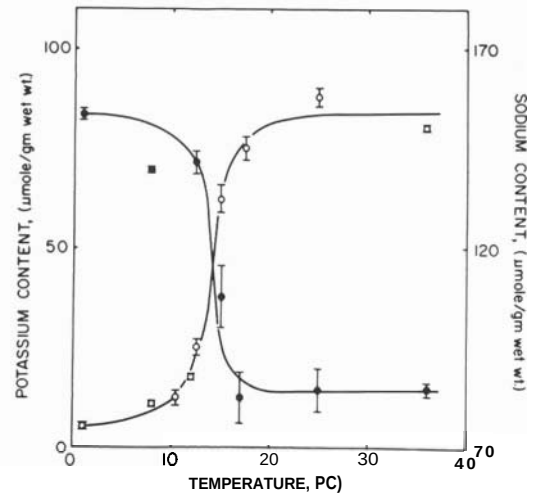

FIGURE 1. **Effect of temperature on potassium (O) and sodium (●) steady state contents of *Taenia coli*. The points are means of 6-16 determinations ± S.E.M. in three different experiments. The high Na content above 17°C is due mainly to the large extracellular space in smooth muscles. The solid lines are arbitrarily drawn to connect the experimental points. (Reisin and Gulati, 1973, by permission of the *Ann.* NY *Acad. Sci.)***

$$[K^+]_{cell} = \alpha q_K [K^+]_{ex} + \frac{[f]}{2} \left\{ 1 + \frac{\left(\frac{[K^+]_{ex}}{[Na]_{ex}} \cdot K^{oo}_{Na\to K} - 1\right)}{\sqrt{\left(\frac{[K^+]_{ex}}{[Na^+]_{ex}} \cdot K^{oo}_{Na\to K} - 1\right)^2 + 4\theta K^{oo}_{Na\to K} \cdot \frac{[K^+]_{ex}}{[Na^+]_{ex}}}} \right\}, \quad (1)$$

and

$$[Na^+]_{cell} = \alpha q_{Na} [Na^+]_{ex} + \frac{[f]}{2} \left\{ 1 - \frac{\left(\frac{[K^+]_{ex}}{[Na]_{ex}} \cdot KE\text{-}K - 1\right)}{\sqrt{\left(\frac{[K^+]_{ex}}{[Na^+]_{ex}} \cdot K^{oo}_{Na\to K} - 1\right)^2 + 4\theta K^{oo}_{Na\to K} \cdot \frac{[K^+]_{ex}}{[Na^+]_{ex}}}} \right\}, \quad (2)$$

where $[K^+]_{cell}$ and $[Na^+]_{cell}$ are the intracellular K and Na^+ concentrations in units of mmoles/kg fresh cells. $[f]$ is the concentration of fixed anionic sites in the cells and is in the same unit. α is the water content in liter/Kg fresh weight. q_K and q_{Na} are the average equilibrium distribution coefficients of K and Na' in the cell water. $[K^+]_{ex}$ and $[Na^+]_{ex}$ are the molar concentrations of K and Na^+ in the surrounding medium in equilibrium with the cells. $K^{oo}_{Na\to K}$ is the intrinsic equilibrium constant of adsorbed K over that of adsorbed Na^+. θ is equal to $\exp(\gamma/RT)$, where $-\gamma/2$ is the nearest neighbor interaction energy. $-\gamma/2$ equals the extra energy involved each time a new K^+–Na^+ pair occurs on nearest neighboring pair of sites on a chain of adsorption sites of cell proteins. R and T are the gas constant and absolute temperature respectively. When $-\gamma/2 > 0$, the reaction is ***autocooperative.*** It signifies that if one site

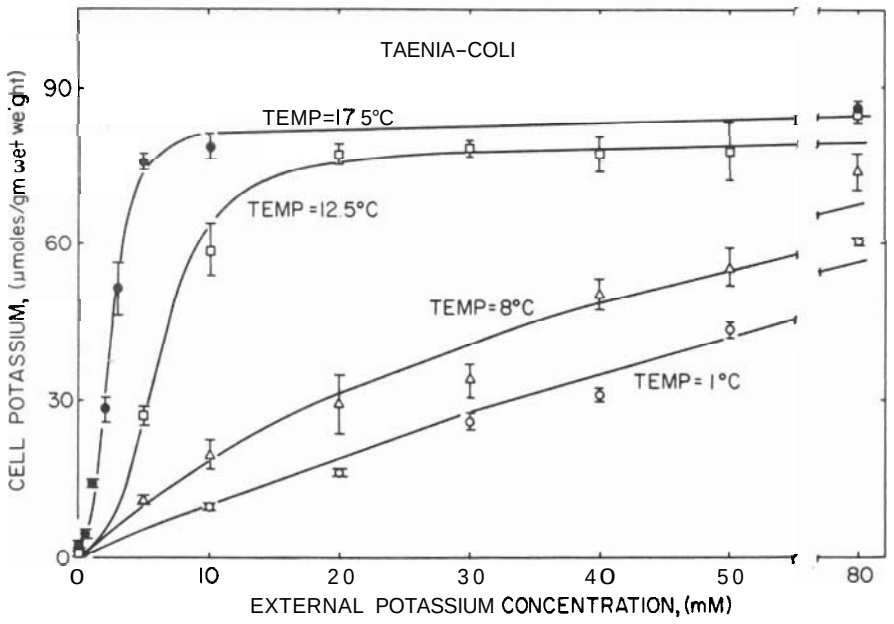

FIGURE 2. Effect of external potassium on cellular potassium content at different temperatures. The solid lines are calculated according to the adsorption isotherm shown in Equation 1 and K^{∞}_{Na-K} and $-\gamma/2$ given in Table I (Reisin and Gulati, 1973, by permission of *Ann.* NY *Acad. Sci.*)

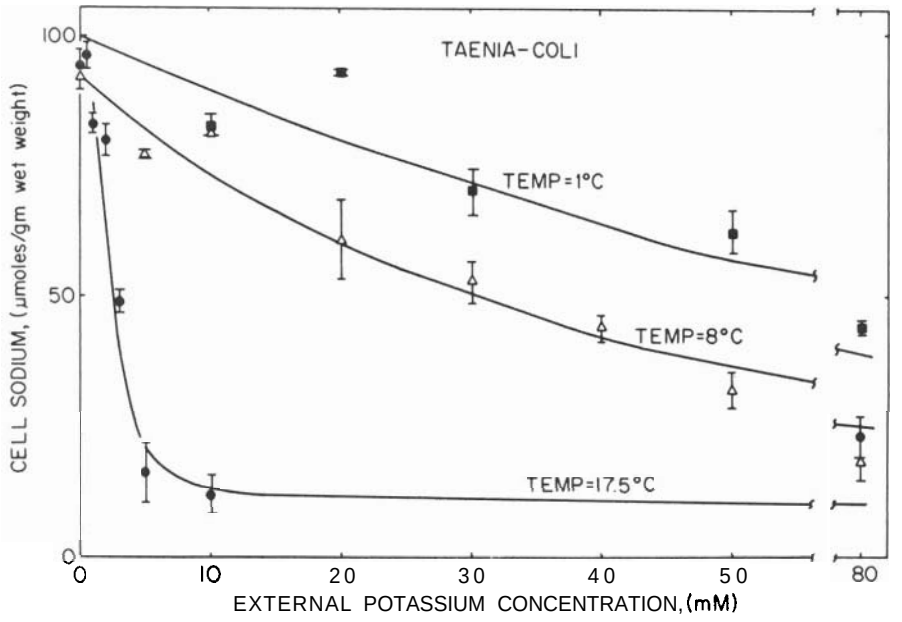

FIGURE 3. Effect of external potassium on cellular sodium content at different temperatures. The solid lines are calculated to the adsorption isotherm shown in Equation 2 and K^{∞}_{Na-K} and $-\gamma/2$ given in Table 1 (Reisin and Gulati, 1973, by permission of *Ann.* NY *Acad. Sci.*)

adsorbs K, this adsorption will make the two nearest neighboring sites prefer K' over Na^+ more than if the middle site is occupied by Na^+. All-or-none shifts from K^+ to Na' states are characteristic of ***autocooperative*** phenomena, in which there is strong interaction between neighboring sites (Ling, 1980).

Figure 1, taken from Reisin and Gulati (1973), shows the effect of temperature change on the K' and Na' distribution in the guinea pig taenia coli (narrow bands of smooth muscle running outside, and along the length of the intestine). Note that the K' and Na' concentrations abruptly changed with cooling from the normal pattern of high K'-low Na^+ to one of low K'-high Na', all within a narrow range of temperature of about 5°C. Outside this temperature transition zone, there is little change of the K' and Na^+ contents with further cooling or warming until irreversible changes set in. Thus if one measures the Q_{10} of say, K' or Na^+ distribution, one would find seemingly contradictory results: very low Q_{10} if the temperature studied was between 25 and 35°F; very high Q_{10} if the temperature studied was between 10° and 20°C, in a manner reminiscent of what Kerkut and Ridge found in snail neurones mentioned above.

Figures 2 and 3 show the cooperative transitions of guinea pig taenia coli in a different way: the K' and Na' distributions were examined at different temperatures in the presence of varying concentration of external K^+ concentration (while the Na' concentration was held constant at 121 mM). Each of the solid lines are theoretical curves calculated according to Equation 1 (Figure 2) and Equation 2 (Figure 3). As the temperature decreased beyond 17.5°C, the curves began to shift to the right corresponding to a decrease of $K^{oo}_{Na\to K}$ (Table I). This temperature change-induced variation of $K^{oo}_{Na\to K}$ is the main reason for the observed K' vs. Na^+ transition. Note that at the normal concentration of K' and Na' in the external medium (K^+, 5 mM; Na', 121 mM), the taenia coli shifted from being fully loaded with K^+ to being loaded with Na^+, in response to a 16.5° fall of temperature from 17.5' to 1°C. Just as noteworthy, was the concomitant fall of the value of $-\gamma/2$. At 17.5°C, $-\gamma/2$ of the best-fitting curve is 0.65 Kcal/mole; at 1°C the curve fitting the data corresponds to a $-\gamma/2$ of 0 Kcal/mole. In other words, autocooperativity seen at the higher temperatures has disappeared at the low temperature beyond the transition temperature.

Temperature Transition of the Resting Potentials. Now according to the AI hypothesis, the behavior of the entire cell including that

Temperature (°C)	[f] (mmoles/kg)	$K^{oo}_{Na\to K}$	θ	$-\gamma/2$ (Kcal/mole)	n
36	85	135	0.11	0.65	3.0
17.5	85	58.9	0.11	0.65	3.0
12.5	80	22.8	0.11	0.65	3.0
8	92	4	0	0	1.0
1	90	18	0	0	1.0

TABLE I. Effect of temperature on parameters of the Yang-Ling isotherm in taenia coli. [f] is the concentration of adsorption sites for K^+ and Na^+. $K^{oo}_{Na\to K}$ is the intrinsic equilibrium constant for the $Na^+ \rightarrow K^+$ exchange; n, the empirical Hill coefficient, is equal to $\exp(\gamma/RT)$; θ is equal to $1/n^2$, (from Reisin and Gulati, 1973 by permission of Ann. NY *Acad. Sci.)*

of all its component parts, are expressions of the properties of basically similar protein-ion-water systems maintained at a high energy state (Ling, 1984, Chapter 6). One of these component parts is the cell surface. If this concept is correct, Equations 1 and 2 should apply to K^+ and Na^+ distribution at the cell surface as well as the whole cell. Unfortunately this expectation is difficult to test directly, due to the extreme thinness of the cell surface and its labile metastable state. However, what cannot be studied directly can be studied indirectly. Thus, according to the surface adsorption theory, K^+ and Na^+ adsorption at the cell surface reveals itself in the magnitude and polarity of the resting potential, ψ, as follows (Ling, 1979, 1982, 1983, 1984):

$$\psi = \text{constant}_1 - RT/F\, \ell n\, ([K^+]_{ex})^{-1} \left\{1 + \frac{(\frac{[K^+]_{ex}}{[Na^+]_{ex}} \cdot K^{oo}_{Na\to K} - 1)}{\sqrt{(\frac{[K^+]_{ex}}{[Na^+]_{ex}} \cdot K^{oo}_{Na\to K} - 1)^2 + 4\theta K^{oo}_{Na\to K} \cdot \frac{[K^+]_{ex}}{[Na^+]_{ex}}}}\right\}. \quad (3)$$

Since Equation 3 contains the same Yang-Ling cooperative adsorption isotherm as in Equations 1 and 2, naturally one may expect that in those tissues where the bulk phase K', Na' distribution undergoes a temperature transition, the K^+ and Na' adsorption and hence the resting potential, which depend on these adsorptions, may also undergo temperature transitions. In that case, one may expect that before cooling, the resting potential will respond to variation of external K' concentration in a manner predicted by Equation 3 with relatively high values of both $K^{oo}_{Na\to K}$. Cooling beyond the transition temperature, will alter the resting potential in such a way that it will still be described by Equation 3 but now with much lower value of $K^{oo}_{Na\to K}$ and $-\gamma/2$. Indeed if the cooling reached as low as or close to O°C, one may expect $-\gamma/2$ to become zero. With $-\gamma/2 = 0$ and thus $\theta = 1$, Equation 3 will then reduce to the much simpler form, first introduced in 1955 (Ling, 1955, 1960, 1962):

$$\psi = \text{constant}_2 + RT/F\, \ell n \left\{\widetilde{K}_K\, [K^+]_{ex}\right\} + \widetilde{K}_{Na}\, [Na^+]_{ex}\}, \quad (4)$$

where $\widetilde{K}_K$ and $\widetilde{K}_{Na}$ are the adsorption constants in units of $(M)^{-1}$ rather than pure numbers as in $K^{oo}_{Na\to K}$ which of course is equal to $\widetilde{K}_K/\widetilde{K}_{Na}$. Equation 4 is an expression of

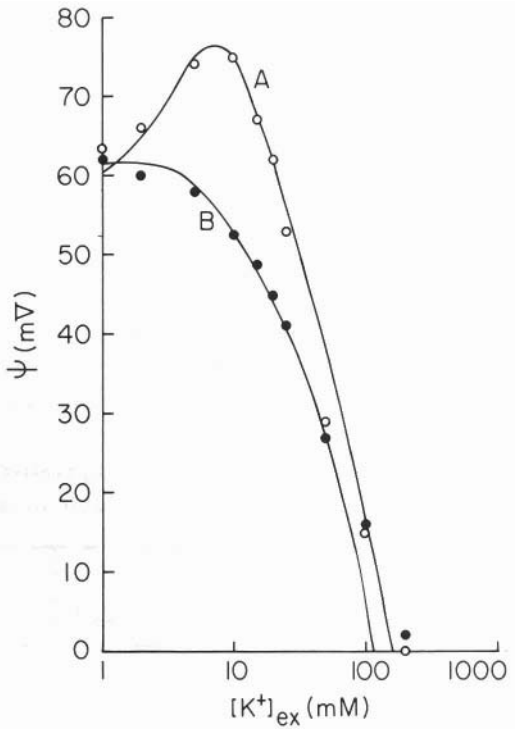

FIGURE 4. Effect of temperature on the resting potential of molluscan neurones at varying external K^+ concentrations. Each point represents the average of 5 experiments. Data from Gorman and Marmor (1970). Standard error of the means, usually less than 5 mV, are not represented. Empty circles (17°C); solid circles (3-4°C). Curve A calculated from Equation 3 with $K^{oo}_{Na\to K}$ = 64 and $-\gamma/2$ = 0.45 Kcal/mole (θ = 0.213) constant, = 93.2 mV; Curve B with $K^{oo}_{Na\to K}$ = 37.6, $-\gamma/2$ = 0 Kcal/mole (θ = 1) and constant, = 66.4 mV.

the surface adsorption theory in its simplest form. To test these predictions requires experimental data of @ in the presence of varying external K' concentrations at temperatures above and below the critical temperature.

There are already at least two sets of excellent studies in the literature that lend themselves to this type of analysis. The first is the work of Gorman and Marmor on snail neurones (1970). In Figure 4, I replotted their data points (their standard errors are not shown). The empty circles represent ψ measured at 37°C. The solid line A going through or near the experimental points was theoretically calculated according to Equation 3 with K^{∞}_{Na-K} equal to 64 and $\theta = 0.213$ corresponding to a $-\gamma/2$ of 0.45 Kcal/mole. The solid circles represent ψ measured at 4°C. The line B is also calculated according to Equation 3 but with K^{∞}_{Na-K} equal to 37.6 and $8 = 1$ corresponding to a $-\gamma/2$ of 0 Kcal/mole. There is also a change in the value of "constant~" (see legend of the figure). For a full discussion of the significance of constant$_1$, see the preceding companion paper (Ling et al., 1984).

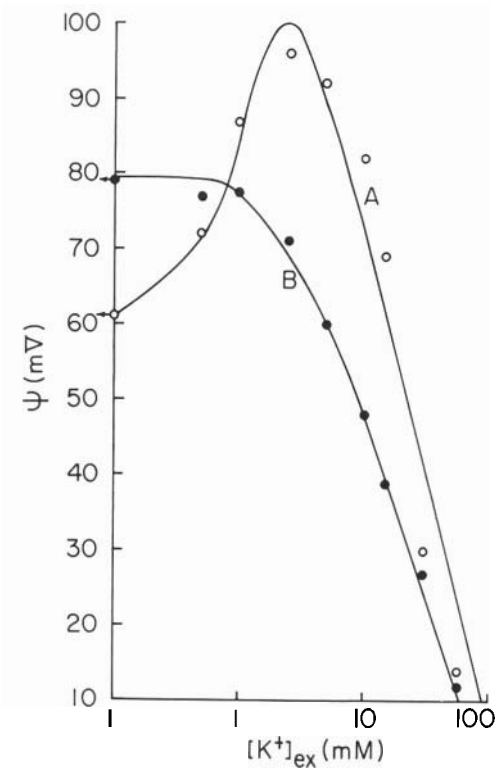

FIGURE 5. Effect of temperature on the resting potential of rat soleus muscle at varying external K^+ concentration. Each point represents average of 30 to 312 muscle fibers in 8 to 30 muscles. Curve A (empty circles) at 37°C; Curve B (solid circles) at 3-4°C. Data points were those of Akaike (1975). Curve A was calculated according to Equation 3 with K^{∞}_{Na-K} equal to 93.3, $-\gamma/2$ = 0.68 Kcal/mole ($\theta = 0.1$), constant, = 116 mV; Curve B with K^{∞}_{Na-K} equal to 46.7, $-\gamma/2 = 0$ Kcal/mole ($\theta = 1$) and constant$_1$ = 84.4 mV.

The second set of data examined was that of Akaike (1975) on soleus muscle preloaded with Na' from rats fed on a diet low in K. (The companion data obtained after treatment with ouabain has already been analyzed in the preceding paper, Ling, et al., 1984.) Here, the empty circles in Figure 5 were obtained by Akaike at 37°C and the line A going through or near most of the experimental points was calculated according to Equation 3 with K^{∞}_{Na-K} equal to 93.3 and $\theta = 0.1$ corresponding to a $-\gamma/2$ of 0.67 Kcal/mole. The solid circles were those obtained after exposure of the soleus muscle to 3 to 4°C. Line B which goes through or near most of the experimental points was calculated according to Equation 3 but with K^{∞}_{Na-K} equal to 46.7 and $8 = 1$ corresponding to $-\gamma/2$ of 0 Kcal/mole. Constant$_1$ was 116 mV for Curve A and 84.4 mV for Curve B.

In conclusion, one finds that the theory of a temperature transition similar to those already demonstrated in the bulk phase K' and Na' adsorption, can adequately describe the resting potential of both snail neurones and rat soleus muscle. In each case the "hyperpolarization" observed in response to a warm temperature reflects a warming-induced increase in K^{∞}_{Na-K}, "constant$_1$" and $-\gamma/2$; conversely cooling had the opposite effects. One may anticipate that a similar explanation applies to all the cells that have been shown to exhibit marked, reversible change of ψ in response to changes of temperature.

Is There a Demonstratable Reversible Temperature Transition in Frog Muscles at Above-Freezing Temperature? I now return to the squid axon and frog muscle mentioned earlier. In contrast to snail neurones and mammalian muscles, the resting potentials of the squid axon and frog muscle do not exhibit significantly different ψ's with change of temperature between 0° to 30°C, beyond that due to the change of the absolute temperature. One naturally thinks of the basic differences between the poikilotherms of which the frog and squid are examples, and the homeotherms which include mammals. While most homeotherms cannot withstand, for long, a body temperature of O°C, poikilotherms like frogs and squids may function at least adequately at a temperature near freezing. On this point, the following quotation from Albert Szent-Gyorgyi is relevant. After presenting theoretical reasons why rabbit muscle cannot contract at 0°C, he remarked, "It was a rather unpleasant surprise one day to find a frog swimming in ice water at 0°C ..." (Szent-Gyorgyi, 1948, p. 64). One asks, Could a frog still swim if its muscles, behaving like guinea pig taenia coli, have lost all their K^+ at O°C? It seems unlikely. Yet it was my turn to be unpleasantly surprised to read in the paper of Reisin and Gulati — from which Figures 1 to 3 as well as Table I were taken — that frog muscle also underwent a temperature transition at above O°C temperature. Their data indicates that while $-\gamma/2$ remained uncanged between 25° and O°C, K^{∞}_{Na-K} dropped from 110 at 25°C to 35 at 0°C.

A careful reading revealed that they obtained their data by way of a "shortcut". In

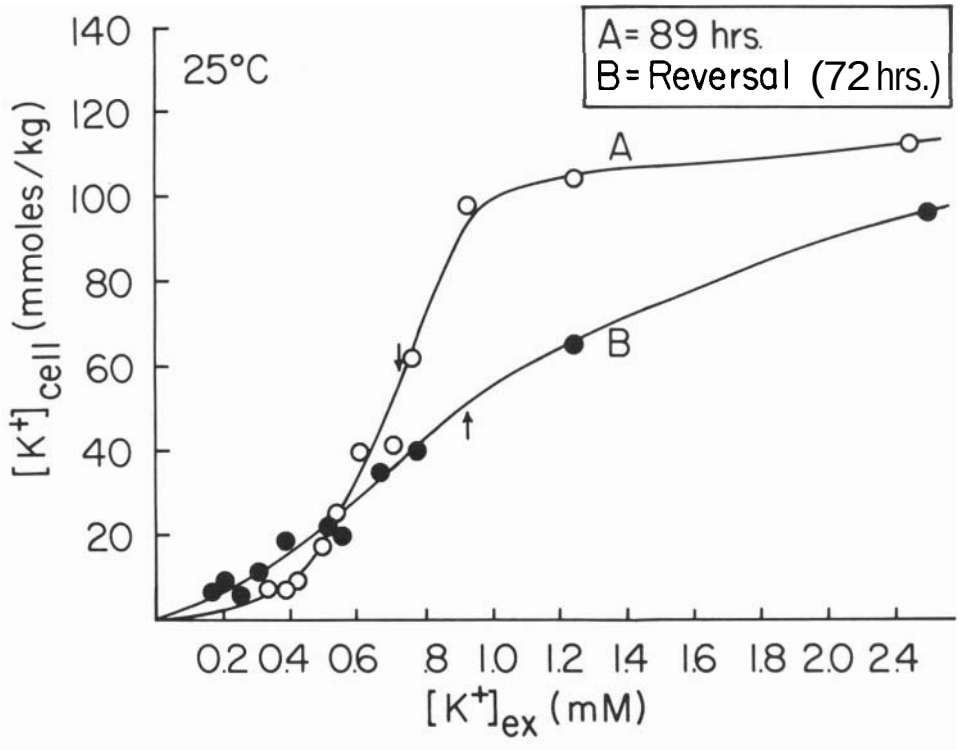

FIGURE 6. Equilibrium distribution of K^+ in frog voluntary muscles in Ringer solution at 25°C containing a constant concentration of Na^+ (100 mM) and varying K^+ as indicated on the abscissa. Curve A (empty circles) was obtained after incubation in the Ringer for 89 hours at 25°C. Curve B (solid circles) was obtained after first incubating all the muscle in a K^+-free Ringer solution for 72 hours at 25°C followed by another 72 hours of incubation in Ringer solution. (For further experimental details, see Ling and Bohr, 1970.)

order to shorten the incubation time for new equilibrium to be reached at very low external K' concentration, they loaded the muscles first with Na^+ by incubation in a K-free Ringer solution at 25°C. They then equilibrated these muscles for 48 hours at 0°C in Ringer solution containing varied but higher K' concentrations before analyzing for K' and Na^+ contents. I suspect that 48 hours might be too short to allow true equilibrium to be reestablished. My reasons and evidence supporting them are as follows:

Ling and Bohr (1970) showed that frog muscle took 72 hours at 25°C to reach new equilibrium in an environment containing the usual concentration of Na' but low K'. Reversal was achieved in about the same length of time when the external K' was restored to normal (2.5 mM). However, whether this was also true when the external K' concentration in the reversal solution was below 2.5 mM, had not been carefully studied previously. Figure 6 shows the result of a new attempt to answer this question. A is a control in which the K' concentrations in the cells were determined after 89 hours of incubation at 25°C in a medium containing a constant concentration of Na^+ (100 mM) and varying concentrations of K' as indicated on the abscissa. It is to be noted that the external concentrations are presented in an expanded scale to reveal small differences. B is from a set of muscles which had been incubated in a large volume of "K-free" solution at 25°C for 72 hours to remove virtually all cell K, followed by a further incubation at 25°C in Ringer solutions containing different concentrations of K' and a constant concentration of Na^+ for another 72 hours. If the data are presented on a condensed scale, shown in Figure 2, the differences between A and B may be hardly noticeable. The expanded scale revealed that even after 72 hours and at the high temperature of 25°C, the reversal was not complete. Now K^{∞}_{Na-K} is equal to $[Na^+]_{ex}/[K^+]_{ex}$ at the point where half of the adsorption sites are occupied by K' and the other half by Na' (as pointed out by arrows in the figure). Thus from the control experiment, K^{∞}_{Na-K} is equal to $100/0.72 = 139$; from the reversal experiment it is equal to $100/0.82 = 122$. These data indicate that the reversal from "K^+-free" to a different but still low external K' concentration may need a longer period of time than that required for similar reversal at much higher external K^+ concentrations. This would be especially true if the reversal was carried out at 0°C and then only for 48 hrs. as was the case in Reisin and Gulati's study.

Finally, I eliminated the reversal step altogether and went ahead directly by incubating the muscles at O°C in Ringer solution containing different K' concentrations and a constant 100 mM of Na^+ at 0°C for 14 days. The result is shown as Curve A of Figure 7 (solid circles). There is little question that equilibrium had been reached to the same extent as at 89 hours of incubation at 25°C since Curve A of Figure 6 and Curve A of

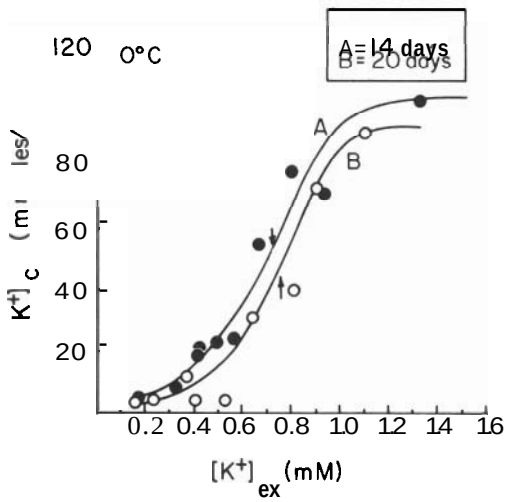

FIGURE 7. Equilibrium distribution of K^+ in frog voluntary muscles after incubation in Ringer solution at 0°C containing a constant concentration of Na^+ (100 mM) and varying external K^+ concentration as indicated on the abscissa. Solid circles and Curve A obtained after 14 days of incubation; empty circles and Curve B after 20 days of incubation.

Figure 7 are almost the same. The $K^{oo}_{Na\to K}$ at 0°C after 14 days of equilibration at 0°C is $100/0.71 = 141$ in comparison with a $K^{oo}_{Na\to K}$ of 139 from Curve A of Figure 6. We incubated another group of frog muscles at O°C for 20 days; the results are shown as open circles in Figure 7. Here the $K^{oo}_{Na\to K}$ of the best fitting curve is equal to $100/0.75 = 133$. Neither 141 nor 133 is significantly different from the value of 139 obtained after 86 hours of incubation at 25°C. These data led me to the conclusion that muscles from frogs captured in Vermont survive well at 0°C. Neither their K^+ nor Na' contents are significantly altered by cooling to O°C.

The absence of a demonstrable reversible temperature transition in the bulk phase K' and Na' distribution in frog muscle is thus in full accord with the similar absence of observable temperature-dependent changes of the resting potential of frog muscle (beyond that due to absolute temperature) mentioned earlier, indicating a lack of temperature transition of K' and Na^+ adsorption at the cell surface as well. As pointed out earlier the resting potential of squid axons shows a similar insensitivity to temperature change. Based on these facts, one may be inclined to think that, in all cells, the electrical potentials have a "single" common origin and that the differences observed between cells, arising perhaps out of evolutionary adaptation to the cell's environment, reflects only differences in the details of quantitative parameters involved, and not in the basic mechanism.

The foregoing work was supported by Office of Naval Research Contract N00014-79-C and NIH Grants 2-R01-CA16301 and 2-R01-GM11422-13.

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(Received September 21, 1984: revised November 13, 1984).