

EFFECTS OF ADRENALINE, CALCIUM, AND OUABAIN ON THE RESTING POTENTIAL OF FROG MUSCLE: INTERPRETATION BASED ON THE THEORY OF ALLOSTERIC CONTROL OF COOPERATIVE INTERACTIONS AMONG SURFACE ANIONIC SITES

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- *The surface adsorption theory of the cellular electric potential, a subsidiary of the association-induction hypothesis, can offer quantitative interpretations of the equilibrium resting potential of frog muscle in the presence of a constant concentration (100 mM) of external Na^+ and varying external K^+ (ranging from 0.1 mM to 100 mM) both in the absence and in the presence of cardinal adsorbents (ouabain, adrenaline, or Ca^{++}). The theory can also quantitatively describe experimental data published by other laboratories from the studies of various cell types in the presence of these and other cardinal adsorbents, including some well known data which, up to now, have been regarded as specific evidence in support of the electrogenic pump theory.*

INTRODUCTION

The Hodgkin-Katz equation of the cellular electrical potential predicts direct dependence of the potential (ψ), (1) on the absolute temperature and (2) on the logarithm of external K^+ and Na^+ concentrations. By and large, these predictions have been confirmed (see below). The Hodgkin-Katz equation also predicts dependence of ψ (3) on the logarithm of external Cl^- concentration, which was not confirmed; and (4) on the logarithm of the intracellular K^+ concentration, which was confirmed by some laboratories but not by a majority of other laboratories (see Ling, 1960, 1982).

In the ionic theory, the differences in the sensitivity of the resting potential (ψ) to various external cations were attributed to differences in their permeability through the cell membrane (for evidence against this concept, see Edelmann, 1973). Thus the greater depolarizing effect of K^+ than Na^+ was attributed to a higher K^+ permeability. However, by itself, a difference in permeability cannot

explain why the cell maintains permanently a much higher steady level of K^+ than Na^+ . For this, the Na^+ pump was postulated to maintain both a K^+ and a Na^+ concentration gradient across the cell surface by constantly pumping Na^+ out and K^+ in. For reasons described below, this kind of Na^+ pump was retroactively called an electrically neutral Na^+ pump.

A second kind of Na^+ pump is called an electrogenic pump. It was postulated in order to explain the quantitative difference between ψ actually measured and that predicted by the Hodgkin-Katz equation (Kernan, 1970; Koketsu, 1971; Thomas, 1972). A prominent example that prompted the introduction of this additional pump was the observed ψ higher (referred to as hyperpolarization) than what the Hodgkin-Katz equation predicted, when frog muscle depleted of its K^+ following prior exposure to a low K^+ — high Na^+ Ringer solution, was returned to a normal Ringer solution containing a normal level of K^+ . It was postulated that under these circumstances, an excess electric charge separation

was achieved and maintained by an electrogenic Na pump which "extrudes more Na' than K' taken up" (Thomas, 1972, p. 567). Amongst the most persuasive evidence for this theory were the observed effects of ouabain and of low temperature on the resting potential in molluscan neurones (Gorman and Marmor, 1970). (For other supportive evidence see Discussion below). Thus the resting potential of the molluscan neurones, which did not follow the Hodgkin-Katz equation, did so after exposure to ouabain and on cooling — both treatments believed to inhibit the postulated electrogenic Na' pump. These observations led Gorman and Marmor to conclude that the resting potential of molluscan neurones has both an ionic component which follows the ionic theory and a metabolic component which does not. Akaike (1975), who studied K⁺-depleted rat soleus muscle, reached a similar conclusion. In the following pages both sets of data will be discussed again in more detail.

From 1977 on, one has witnessed the rapid accumulation of mutually supportive experimental evidence showing that the bulk of intracellular K' in frog and insect voluntary muscle cells is not free but is in an adsorbed state on sites located primarily at the edges of the A bands and at the Z-lines (Edelmann, 1977, 1980, 1983; Ling, 1977, 1984; Trombitas and Tigy-Sebes, 1979). These unanimous findings from studies using four kinds of widely divergent techniques and by three different laboratories have disproved a basic tenet of the membrane-pump theory, i.e., the tenet that cell K' is free. Since both Bernstein's membrane theory of the cellular electric potential and Hodgkin and Katz's ionic theory are built on the assumption that the bulk of cell K^t is free and that the potential is the consequence of the diffusion of the (free) cell K⁺, it has become necessary to consider a different theory of the cellular electric potential that is compatible with the newly established adsorbed state of the bulk of cell K' as

well as a host of other evidence against the membrane-pump theory that has accumulated (Ling, 1984a).

In fact, such a theory has been in existence for some time, bearing the name, the surface adsorption (SA) theory (Ling, 1955, 1960, 1962, 1982, 1983, 1984a). It has been shown that the SA theory, besides being compatible with the adsorbed state of cell K', agrees with other experimental observations incompatible with the ionic theory. Thus, as part of the association-induction (AI) hypothesis (Ling, 1984a) it obviates the difficulty created by the insufficient energy to operate the Na pumps and other pumps, (see below). In this communication, we shall demonstrate that the SA theory can offer reasonable interpretations of those experimental findings that had been thus far explained almost exclusively in terms of the electrogenic pump theory, as well as new experimental findings to be described.

THEORY

According to the AI hypothesis, the coherent behavior of living cells rests upon the ability of protein chains to transmit energy and information over long distance by the propagated short-range inductive effects between nearest neighboring "sites" along the length of the polypeptide chain. In this hypothesis the selective accumulation of K' over Na' in living cells is not due to membrane pumps but reflects two basic mechanisms: preferential adsorption of K' over Na⁺ on the β - and γ -carboxyl groups of cell proteins and partial exclusion of hydrated Na⁺ (and K^t) from the bulk of cell water which exists in polarized multilayers on extended polypeptide chains of certain cell proteins called "matrix proteins". It was shown that K' (and Na⁺) accumulation in frog muscle and a variety of other living cells can be described by Ling's general equation for solute distribution (Ling, 1965) which includes the Yang-

Ling cooperative isotherm describing the adsorbed solute in the cell. For the specific case of K^+ (and Na^+) distribution, this equation assumes the following form:

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

where $[K^+]_{ex}$ is the external K^+ concentration in molarity. α is the percentage water content. q_K is the (average) equilibrium distribution coefficient of K^+ between cell water and water in the external bathing medium. $[K^+]_{cell}$ is the K^+ concentration and $[f]$, the total concentration of anionic adsorption sites in the cell, both in units of mmoles per kilogram of fresh cells. ξ and θ are defined as follows:

$$\xi = \frac{[K^+]_{ex}}{[Na^+]_{ex}} \cdot K_{Na-K}^{oo}, \quad (2)$$

where K_{Na-K}^{oo} is the intrinsic equilibrium constant for the Na^+ and K^+ exchange. $[Na^+]_{ex}$ is the external Na^+ concentration;

$$\theta = \exp(\gamma/RT), \quad (3)$$

and $-\gamma/2$ is the nearest neighbor interaction energy (see Ling, 1964, 1970).

In the AI hypothesis, a large number of substances which can exercise powerful influence over physiological behavior of the living cell are called "cardinal adsorbents". They include ATP, Ca^{++} , ouabain, many other drugs, hormones, and biologically active agents. By adsorbing on "cardinal sites" of a key protein, a cardinal adsorbent creates an inductively-propagated allosteric effect on a "gang" of cooperatively linked "regular sites". These regular sites on which K^+ and Na^+ are adsorbed are considered to be primarily the β - and γ -carboxyl groups. In this case the electron density of the singly charged oxygen

atom of the β - and γ -carboxyl groups becomes either increased or decreased as a result of the allosteric effect. In other words the c -value, which measures this electron density, is either increased or decreased. Theoretical calculations showed that such changes of the c -value lead to changes in the values of K_{Na-K}^{oo} mentioned above (Ling, 1962, 1984a).

It has been shown that the Yang-Ling adsorption isotherm on the right hand side of Equation (1) quantitatively describes the autocoperative oxygen binding on hemoglobin in vitro as well as the allosteric control of this oxygen binding by 2,3-diphosphoglycerate, by inositol hexaphosphate, and by ATP which alter the intrinsic equilibrium constant of oxygen binding on the four heme sites (Benesch and Benesch, 1969; Chanutin and Curnish, 1967; Ling, 1970).

It has also been shown that ouabain increases the Na^+ content and decreases the K^+ content of living cells by lowering the value of K_{Na-K}^{oo} and $-\gamma/2$ according to Equation (1) (Ling and Bohr, 1971a; Gulati, 1973; Negen-dank and Shaller, 1982). Furthermore, these effects persist in frog muscle cells whose postulated membrane pumps have been made non-functional in the effectively membrane-pump-less open-ended cell (EMOC) preparations (Ling, 1973, 1978), thus demonstrating that ouabain acts directly on the K^+ and Na^+ adsorbing sites located on the A bands and Z-lines of muscle cells (Edelmann, 1977, 1983; Ling, 1984a) rather than via a postulated Na^+ pump.

In the AI hypothesis, the protein-ion-water system is the universal substance of life whether found at some specific locations of sub-cellular structures inside the cell or at its surface. Thus the electron withdrawing and donating properties of cardinal adsorbents and the resultant change of K_{Na-K}^{oo} and $-\gamma/2$ demonstrated in bulk-phase ion distribution may well be duplicated in protein-ion-water systems that comprise the cell surface, con-

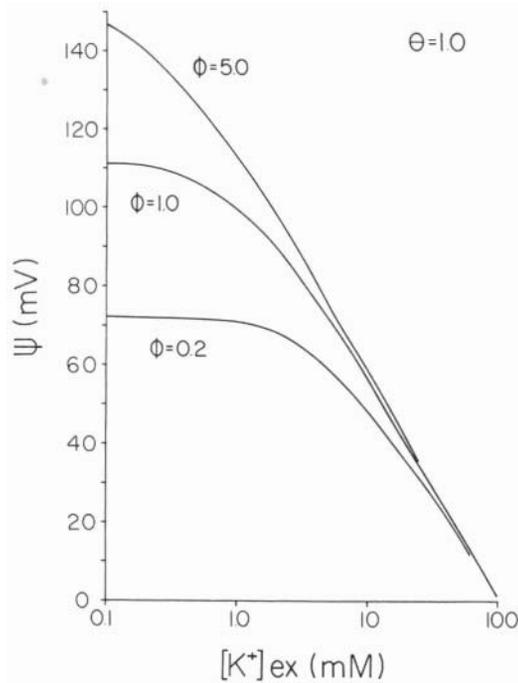


FIGURE 1. Theoretically calculated resting potential (ψ) of living cells in the presence of varying external concentrations of external K^+ , where the nearest neighbor interaction is zero ($-\gamma/2 = 0$ Kcal/mole, $\theta = 1$). In this and following theoretical calculations given in Figures 2 to 4, it was assumed that $\psi = 85$ mV at $[K^+]_{ex}$ of 2.5 mM and that a constant, 100 mM Na^+ is present in the external medium. $\phi = 1$ when $K_{Na-K}^{00} = 100$; $\phi = 5.0$, $K_{Na-K}^{00} = 500$; $\phi = 0.2$, $K_{Na-K}^{00} = 20$. Theoretical values are calculated on the basis of Equation (4).

ventionally referred to as the cell membrane. Since according to the surface adsorption theory of the cellular electrical potential mentioned above, the adsorption of K^+ (and Na^+) at the cell surface anionic sites determines the polarity and magnitude of the cell potential, cardinal adsorbents that affect the bulk phase K^+ and Na^+ distribution may well have parallel effects on the cell potential. An opportunity to test this hypothesis arose after Ling proposed in 1979 a new equation for the cellular electrical potential in which cooperative interactions among the potential-generating ionic sites at the cell surface are considered:

$$\psi = \text{constant}, -\frac{RT}{F} \ln [K^+]_{ex}^{-1} \left\{ 1 + \frac{\xi - 1}{(1 - \xi)^2 + 4\xi\theta} \right\} \quad (4)$$

Ling and Fisher (1983) then showed that long incubation was required for the resting potential of frog muscles to reach equilibrium when the external K^+ concentration was below normal (2.5 mM). When this requirement was taken into account in obtaining ψ at different external K^+ concentrations, the observed equilibrium resting potential dropped to low values at low $[K^+]_{ex}$ according to Equation (4). Such a relation is not predicted by the Hodgkin-Katz equation. In this paper, we shall concentrate on how variations of K_{Na-K}^{00} (and $-\gamma/2$) cause changes of the equilibrium ψ according to Equation (4), and compare these theoretically predicted

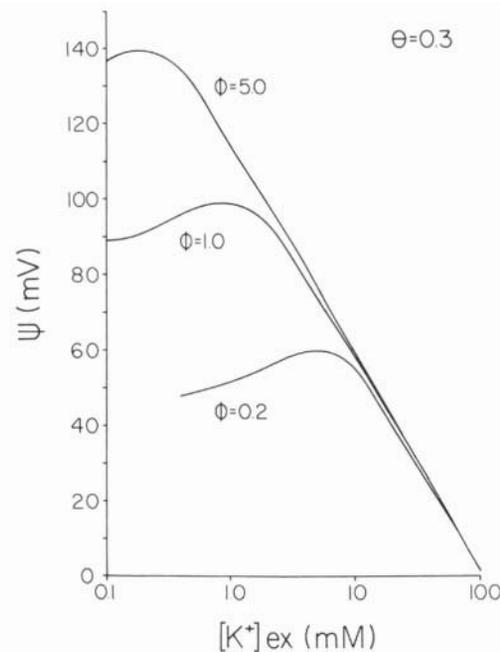


FIGURE 2. Theoretically calculated resting potential (ψ) of living cells in the presence of varying concentrations of external K^+ , where the nearest neighbor interaction energy is 0.35 Kcal/mole ($\theta = 0.3$). Other conditions same as described under Figure 1.

relations with experimentally measured ψ in the presence and absence of cardinal adsorbents.

Except where otherwise stated, we chose the following set of experimental conditions and theoretical parameters: (i) $[\text{Na}^+]_{\text{ex}}$ is kept constant at 100 mM; (ii) ψ is equal to 85 mV when the inside of the cell is negative in reference to the external (grounded) medium at an external K^+ concentration of 2.5 mM; (iii) ϕ is the ratio of $K_{\text{Na-K}}^{\text{oo}}$ in the presence of a drug (or other cardinal adsorbent) over $K_{\text{Na-K}}^{\text{oo}}$ without the drug. Thus we assumed that at $\phi = 1$, $K_{\text{Na-K}}^{\text{oo}} = 100$; at $\phi = 0.2$, $K_{\text{Na-K}}^{\text{oo}} = 20$; at $\phi = 5$, $K_{\text{Na-K}}^{\text{oo}} = 500$.

We calculated ψ according to Equation (4) with different values of θ : Figure 1 ($\theta = 0$, $n = 1$, $-\gamma/2 = 0$ Kcal/mole); Figure 2 ($\theta = 0.3$, $n = 1.82$, $-\gamma/2 = 0.35$ Kcal/mole); Figure 3 ($\theta = 0.1$, $n = 3.2$, $-\gamma/2 = 0.68$ Kcal/mole);

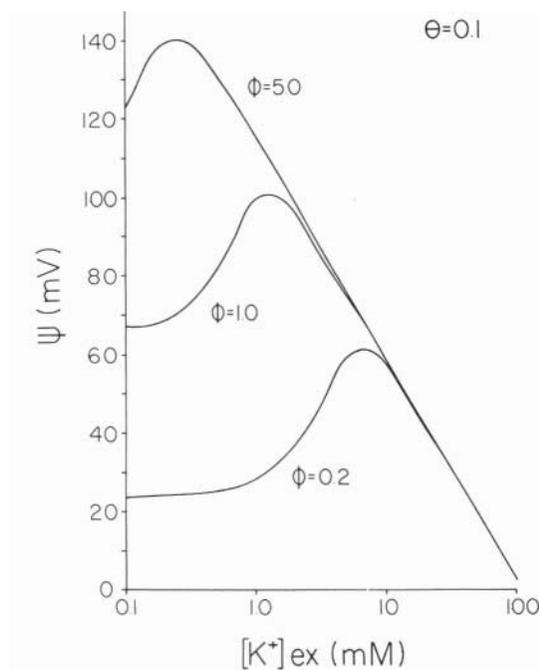


FIGURE 3. Theoretically calculated resting potential (ψ) of living cells in the presence of varying concentrations of external K^+ , where the nearest neighbor interaction energy is 0.67 Kcal/mole ($\theta = 0.1$). Other conditions same as described in Figure 1.

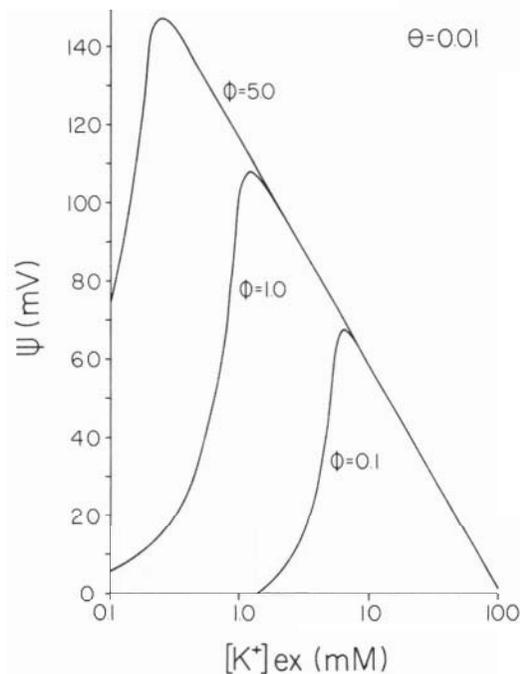


FIGURE 4. Theoretically calculated resting potential (ψ) of living cells in the presence of varying concentrations of external K^+ , where the nearest neighbor interaction energy is 1.35 Kcal/mole ($\theta = 0.01$). Other conditions same as described in Figure 1.

and Figure 4 ($\theta = 0.01$, $n = 10$, $-\gamma/2 = 1.36$ Kcal/mole). In each case three values of $K_{\text{Na-K}}^{\text{oo}}$ were used: 20 ($\phi = 0.2$); 100 ($\phi = 1.0$); and 500 ($\phi = 5.0$). n , the Hill's coefficient, equals $\exp(-\gamma/2 RT)$ (Ling, 1964).

Figure 1 shows that when there is no nearest neighbor interaction ($-\gamma/2 = 0$, $\theta = 0$), ψ flattens out at low $[\text{K}^+]_{\text{ex}}$. This behavior is similar to that predicted by the Hodgkin-Katz equation and by the earlier SA theory represented as Equation (6) below. In the region of low $[\text{K}^+]_{\text{ex}}$ and moderately high $[\text{K}^+]_{\text{ex}}$, ψ rises with increase of θ (or $K_{\text{Na-K}}^{\text{oo}}$). However at very high $[\text{K}^+]_{\text{ex}}$, differences in $K_{\text{Na-K}}^{\text{oo}}$ exercise much smaller effect on ψ .

When $-\gamma/2$ is larger than 0 ($\theta < 1.0$), as in Figures 2 to 4, there are autocoperative interactions among the surface anionic sites. With progressive decrease of $[\text{K}^+]_{\text{ex}}$ below normal (2.5 mM), a point will be reached

where the surface sites suddenly shift from occupancy primarily by K^+ to one primarily by Na^+ . As a result, ψ becomes lower. The abruptness with which the downward turn of ψ takes place (with decreasing external K^+ concentration) increases with the increase of the value of $-\gamma/2$. At $-\gamma/2 = 1.36$ Kcal/mole (Figure 4) the downward drop of ψ becomes quite steep.

An important parameter that is not explicitly represented in Equation (4) is the concentration of fixed ionic sites at the cell surface. According to the SA theory, there is no resting potential if there are no fixed ionic sites on the cell surface. Indeed there is much evidence in model studies for the mandatory requirement of fixed surface ionic sites to create a surface potential (see Ling, 1984a, pps. 108,472). This omission of surface ionic site concentration in Equation (4) came from the simplifying assumptions used in the derivation of the equation (see Ling, 1979). Nevertheless, one can provide indirect evidence that the constant, of Equation (4) does contain a term relating to the surface fixed ionic site concentration. Consider the case where there is no nearest-neighbor interaction among the surface anionic sites (i.e., $-\gamma/2 =$

0, $\theta = 1$) and the fact that $K_{Na-K}^{\circ} = \tilde{K}_K / \tilde{K}_{Na}$ where \tilde{K}_K and \tilde{K}_{Na} are the adsorption constants for K^+ and Na^+ on the surface anionic sites. We can write Equation (4) in the simplified form:

$$\psi = \text{constant} - \frac{RT}{F} \ln (\tilde{K}_K [K^+]_{ex} + \tilde{K}_{Na} [Na^+]_{ex}). \quad (5)$$

Equation (5) is, of course, similar to the original equation for the cellular electrical potential (without cooperative interaction) presented by Ling in 1960:

$$\psi = \text{constant}_2 - \frac{RT}{F} \ln (\tilde{K}_K [K^+]_{ex} + \tilde{K}_{Na} [Na^+]_{ex}). \quad (6)$$

Only here constant_2 is known and is equal to $(RT/F) \ln [f^-]$, where $[f^-]$ is the concentration of the fixed anionic sites at the cell surface as mentioned above. Since Equations (5) and (6) describe the same phenomenon (and are otherwise identical), constant_2 in Equation (6) also includes $(RT/F) \ln [f^-]$.

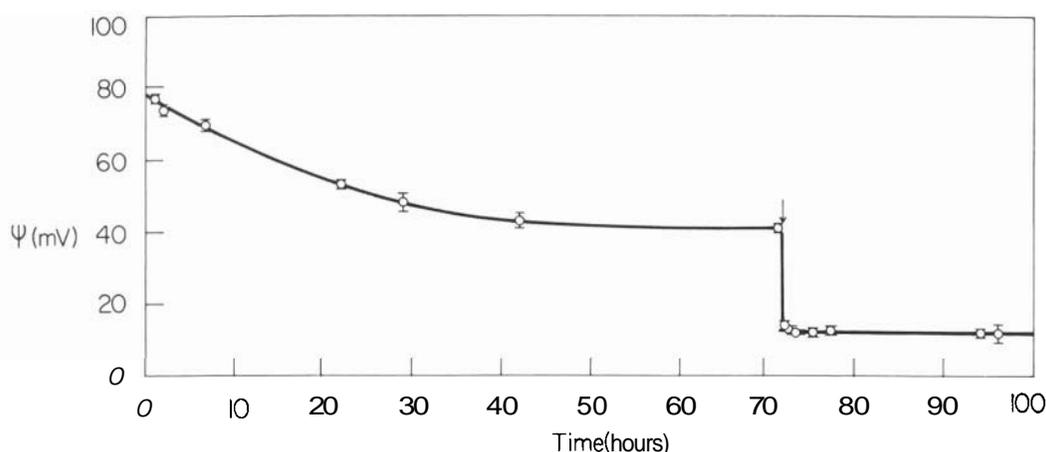


FIGURE 5. The time course of change of the resting potential (ψ) of frog sartorius muscle in 731 Ringer solution containing 3.27×10^{-7} M ouabain at 25°C . The external K^+ concentration was 2.5 mM until the 72nd hour at which time the solution was changed to one containing the same concentration of ouabain but 20 mM K^+ . Each point represents the mean of six readings \pm S.E.

MATERIALS AND METHODS

All experiments were performed on the isolated sartorius muscles of leopard frogs (*Rana pipiens pipiens*, Schreber). Most frogs used were from Vermont but some were from New Jersey. The technique of measuring the resting potential was essentially that described by Ling and Gerard (1949a).

Ouabain, and caffeine were obtained from Sigma Chemical Company, St. Louis, Mo. The Ringer solution used, described as Ringer solution 731, contained the following ingredients in millimolarity: K⁺, 2.5; Na⁺, 100; Ca⁺⁺, 1; Mg⁺⁺, 1.2; Cl⁻, 86.7; HCO₃⁻, 15.7; PO₄, 2.7; NO₃⁻, 0.1; and D-glucose, 23.5. In addition, frog Ringer solution 731 contained 14 vitamins and 21 amino acids and penicillin Na (0.1/mg/ml) and streptomycin (0.1 mg/ml) (for details, see Ling and Bohr, 1969). The Ringer solution was equilibrated with a mixture of 95% O₂ and 5% CO₂ and kept strictly sterile. All incubations were carried out in a constant temperature room main-

tained at 25° ± 1°C in 250 ml Erlenmeyer flasks with gentle shaking (60 excursions/min., each excursion 1 inch).

For Ringer solutions containing less than 2.5 mM K⁺, osmotically equivalent amounts of sucrose were added. For Ringer solutions containing more than 2.5 mM K⁺, additional solid KCl was added to the Ringer solution without other alterations. In all cases, the total Na⁺ concentration was kept constant at 100 mM.

RESULTS

Time Course of Resting Potential Change in Response to Ouabain in Normal and High K⁺ Ringer. Figure 5 shows how the resting potential of frog sartorius muscle declined slowly in a 731 Ringer solution containing 2.5 mM K⁺ and 3.27 × 10⁻⁷ M ouabain until a steady level at 41 mV was reached in about 3 days (25°C). At 71 hours, the bathing solution was switched to a 731 solution containing the same concentration of ouabain (3.27

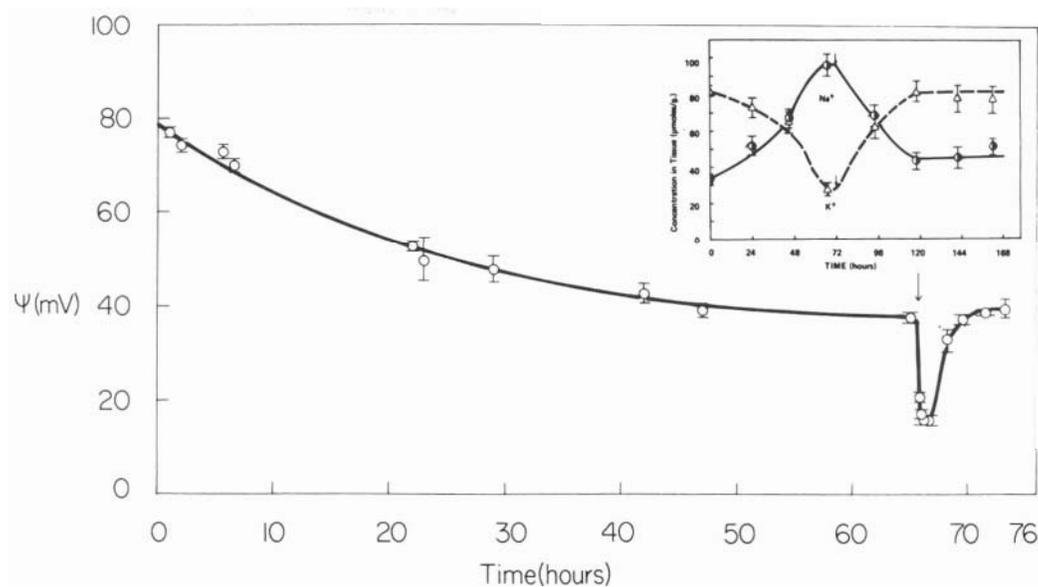


FIGURE 6. The time course of the change of the resting potential (ψ) of frog sartorius muscles in Ringer solution containing ouabain (25°C). The external solution contains 2.5 mM K⁺ and 3.27 × 10⁻⁷ M ouabain until the 66th hour at which time the solution was changed to one containing 20 mM K⁺ but no ouabain. Inset shows the total K⁺ and Na⁺ concentration in similar sartorius muscles treated to the same concentration of ouabain in 731 Ringer solution containing 2.5 mM K⁺. At the 72nd hour, the muscles were moved to a 731 Ringer solution containing no ouabain.

$\times 10^{-7}$ M) but 25.0 mM K. The potential promptly dropped to a new steady level at 12 mV, which was maintained longer than the next 28 hours of continued observation. In general the pattern of response of the resting potential to ouabain resembles that produced by exposure to K^+ -free solution described in a preceding paper (Ling and Fisher, 1983) except that the initial hyperpolarization occurred in the K^+ -free-solution-treated muscles but not in the ouabain-treated ones. The slow attainment of a new equilibrium potential occurred on exposure to simple K^+ -free solution or to Ringer solution containing low or intermediate K^+ concentration and ouabain. Prompt attainment of a new equilibrium potential on exposure to higher external K^+ concentration also occurred in the K^+ -depleted muscles obtained by either K^+ -free solution or ouabain.

The Effect of Removal of Ouabain from the External Solution. Figure 6 describes an experiment similar to that shown in Figure 5, except that the exposure to ouabain lasted only 65 hours, at which time the potential

had reached a steady level of 38 mV. The solution was then changed to 731 Ringer solution containing 25.0 mM KCl but *without* ouabain. The potential abruptly fell to about 16 mV and then gently rose to a new higher level. The time it took to reach this new steady level in the ouabain-free 25.0 mM K^+ Ringer solution was about 6 hours. In the inset we have reproduced from Ling and Bohr (1971a) a time course of change of the total K^+ and Na^+ concentration in frog muscles exposed to a similar concentration of ouabain (3.26×10^{-7} M). It took about the same length of time for the total cell K^+ to fall to a new level (ca. 65 hours at 25°) as it took the resting potential to fall to the new low level. After changing to a ouabain-free Ringer solution, it took almost as long for the total K^+ and Na^+ to return to their original levels, but on returning to a ouabain-free solution, it took only 6 hours for the resting potential to arrive at a new steady level. Within this period of time, the total K^+ concentration of the muscles had risen from 25 $\mu\text{moles/g}$ to no more than 35 $\mu\text{moles/g}$ and thus a long

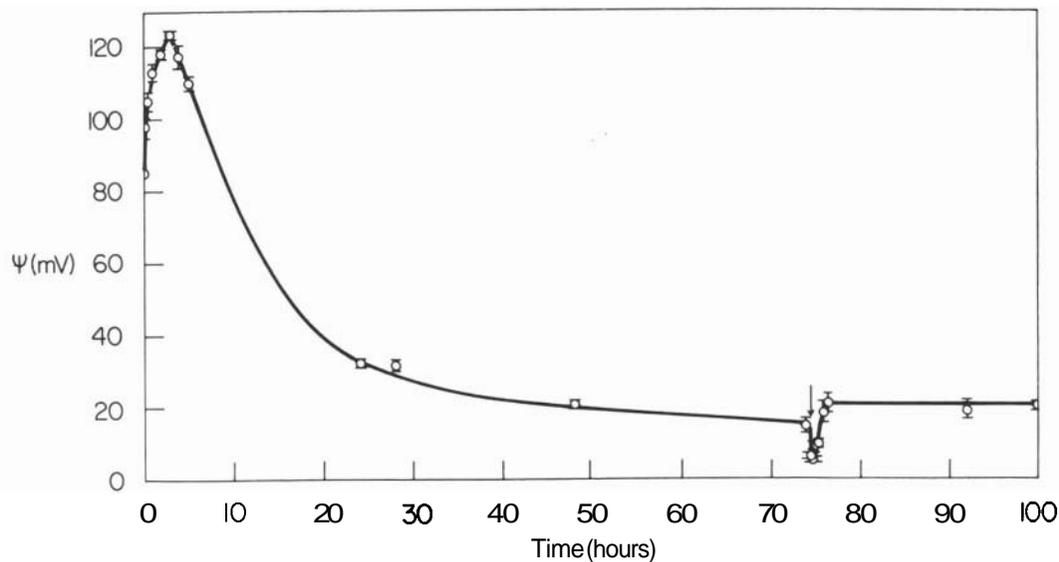


FIGURE 7. The time course of the change of the resting potential of frog sartorius muscle in a " K^+ -free" 731 Ringer solution containing 3.27×10^{-7} M ouabain. At the 74th hour, the bathing solution was changed to a " K^+ -free" Ringer solution containing no ouabain.

way from the 80 $\mu\text{moles/g}$ level it was eventually to attain.

The rapid attainment of a higher equilibrium level of potential in a ouabain-free solution, parallels a similar rapid attainment of a higher equilibrium potential, when muscles which had been kept in a K'-free Ringer solution for 3 days were returned to a normal Ringer solution containing a normal concentration of K' (2.5 mM) (Ling and Fisher, 1983). In both cases, the rapid response suggests that the K' adsorbing sites generating ψ are at or near the cell surface, in support of the SA theory. A sigmoid time course of the potential change would be in accord with similar sigmoid time courses of change of anionic sites from one **autocooperative** (Na') state to another (K') state (Ling, 1964, p. 424; Negendank and Karreman, 1979).

Figure 7 shows the results of an experiment in which the muscle was first incubated for 74 hours in a K'-free solution also containing 3.27×10^{-7} M ouabain and then switched to a 731 Ringer solution containing 25.0 mM KCl but no ouabain. There was a notable initial hyperpolarization to over 120 mV. The final equilibration level of potential (15 mM) in the ouabain-containing K'-free Ringer solution was considerably below those in response to ouabain-containing Ringer solution with normal K' (2.5 mM) shown in Figures 5 and 6 (ca. 40 mV). Similarly, the final equilibrium level after removal of ouabain was also lower in Figure 7 (20 mV) than in Figure 6 (40 mV).

The three sets of data given in Figures 5 to 7 demonstrate that in order to determine the true equilibrium level of the potential of frog muscle after exposure to ouabain, it is not enough to measure the potential after a few minutes or even hours of exposure. Rather one has to expose the muscle for three days (at 25°) to obtain the true equilibrium potentials, except at very high external K⁺ concentration, in which case, a shorter exposure would suffice (see below).

The Resting Potentials at Varying $[\text{K}^+]_{\text{ex}}$ and the Effect of Ouabain on Them. Figure 8 is a composite of three sets of data of normal muscles in which all the muscles exposed to below 2.5 mM K' were first incubated 72 hours in a K'-free Ringer solution and then incubated in Ringer solution containing different $[\text{K}^+]_{\text{ex}}$ as indicated on the abscissa. The resting potentials of all muscles exposed to K' at concentration at and higher than 2.5

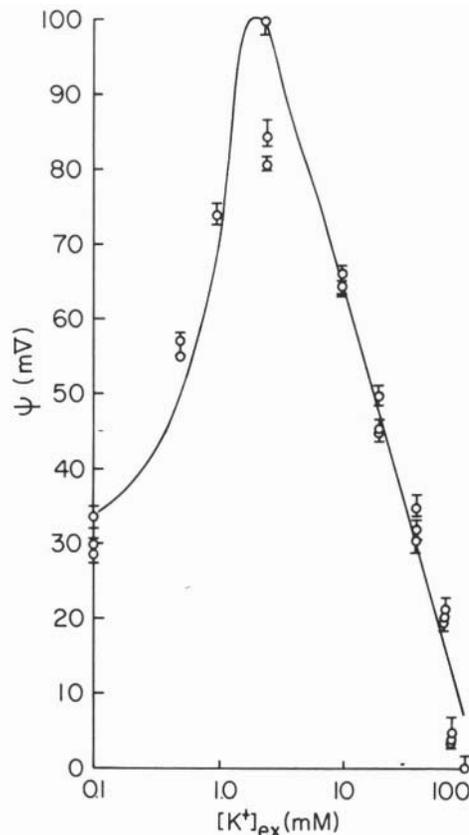


FIGURE 8. The resting potential of frog sartorius muscles in 731 Ringer solution containing 100 mM Na⁺ but varying concentration of K⁺. Combined results of 3 sets of experiments. Each point represents the average of 6 readings \pm S.E. For all K⁺ concentrations lower than 2.5 mM, muscles were incubated in repeated changes of the solution for 3 days (25°C) before measurements were made. For K⁺ concentration equal to or above 2.5 mM, the muscles were incubated for only 15 minutes before measurements. Solid line is theoretical calculated according to Equation (4) and $-\gamma/2 = 0.98$ Kcal/mole ($\theta = 0.035$), $K_{\text{Na-K}}^{\text{O}}$ = 66.7, and constant, = 112 mV.

mM were measured after 15 minutes of incubation in the experimental solutions. The different procedures were chosen because in an external K⁺ concentration higher than 2.5 mM ψ reached new equilibrium almost instantly (Ling, 1960) while muscles in Ringer's solutions containing K⁺ concentration below 2.5 mM took much longer time as shown in Figures 5 to 7 (see also Ling and Fisher, 1983). The line shown in Figure 8 was calculated from Equation (4) with $\theta = 0.035$ ($-\gamma/2 = 1.0$ Kcal/mole) and K_{Na-K}^{∞} equal to 66.7. The nearest neighbor interaction energy at the frog muscle cell surface is thus higher

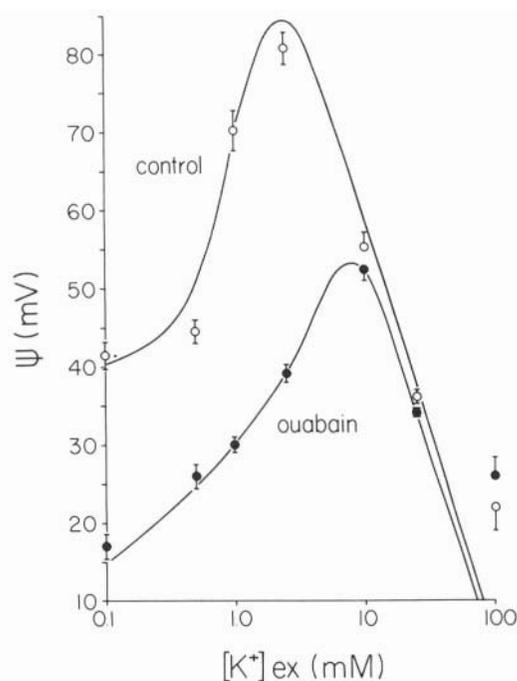


FIGURE 9. Resting potential of frog sartorius muscles incubated for 72 hours (25°C) in 731 Ringer solution containing varying external K⁺ concentration and 3.27×10^{-7} M ouabain (data labelled ouabain) and similarly treated muscles which were then washed and incubated in a large volume of 731 Ringer solution containing the same concentration of external K⁺ but no ouabain (data labelled "control"). Theoretical curves were calculated from Equation (4). For the curve labelled "ouabain" $-\gamma/2 = 0.56$ Kcal/mole ($\theta = 0.15$) and $K_{Na-K}^{\infty} = 12.5$, constant, = 69.7 mV. For the curve labelled "control" obtained after reversal, $-\gamma/2 = 0.73$ Kcal/mole ($\theta = 0.0735$), $K_{Na-K}^{\infty} = 40$, and constant = 100 mV.

than that of bulk phase K⁺, Na⁺ distribution studies at 0.54 Kcal/mole. The intrinsic equilibrium constant, on the other hand is lower than that from the bulk phase studies, which was 135 (Ling and Bohr, 1970).

In Figure 9, the curve labelled ouabain shows the equilibrium resting potential at different $[K^+]_{ex}$ measured after the muscles had been incubated for 70 hours in Ringer solution containing different $[K^+]_{ex}$, ranging from 0.1 mM to 100 mM, all containing 3.27×10^{-7} M ouabain. After the measurements, each muscle was transferred to a Ringer solution containing the same $[K^+]_{ex}$ as the one the muscle had been incubated in but no ouabain. After another 23 hours of incubation, ψ was measured again and the data presented in Figure 8 as "control". The solid lines were calculated from Equation 4 with the following sets of values: For the ouabain muscle $\theta = 0.15$ ($-\gamma/2 = 0.56$ Kcal/mole); $K_{Na-K}^{\infty} = 12.5$. For the "control" after reversal: $\theta = 0.0735$ ($-\gamma/2 = 0.73$ Kcal/mole); $K_{Na-K}^{\infty} = 40$.

A comparison of the "control" after reversal shown in Figures 9 and 8 shows that for most of the data points, reversal was not far from complete, except at $[K^+]_{ex}$ equal to 100 mM. Here the "reversed" control shows a much higher ψ . The reason for this hyperpolarization will be described below. By and large 3.27×10^{-7} ouabain has reduced K_{Na-K}^{∞} by a factor of $66.7/12.5 = 5.3$; it has also reduced $-\gamma/2$ by a factor of $1.0/0.56 = 1.8$. The same concentration of ouabain reduced K_{Na-K}^{∞} of bulk phase K⁺ and Na⁺ distribution in frog muscle cell by a factor of about 5 (Ling and Bohr, 1971). In general, the quantitative concordance between the effects of ouabain on ψ and on the bulk phase K⁺ and Na⁺ distribution confirms the theoretical expectation earlier discussed.

The Time Course of the Response of ψ to Adrenaline. Adrenaline at the concentration of 2.73×10^{-5} M (5 ppm) caused the resting

potential of frog sartorius muscle exposed in normal Ringer solution to rise from 82.3 ± 4.4 (S.D.) to 92.9 ± 3.8 mV after 3 to 5 hrs. of incubation (Ling, 1952). Hyperpolarization created on exposure of living cells to adrenaline has been explained as due to increased membrane permeability to K^+ and Cl^- (Ohashi, 1971), and more recently as due to activation of an electrogenic pump (Morita and Koketsu, 1979). Figure 10 shows the change of ψ of frog muscle on exposure to 3.83×10^{-5} M (7 ppm) of adrenaline. ψ rose from about 80 mV to about 94 mV after 3 hours, in agreement with the above-mentioned earlier report. However, prior to this hyperpolarization, ψ actually dipped sharply at between 10 and 16 minutes followed by a sharp rise to a plateau value of some 86 mV and then a further climb to about 93 mV at about 1 hour. From then on ψ remained more or less constant.

The rapid attainment of equilibrium of the ψ of the adrenaline-treated muscle stands in sharp contrast to the slow attainment of equilibrium of the ψ of ouabain-treated muscle. This different behavior is not difficult to understand. Ouabain, like K^+ -depletion, *decreases* surface adsorption of K^+ ; in both cases the slow release of K^+ from the cell interior delays the attainment of equilibrium. Adrenaline's action is to *increase* K_{Na-K}^{00} of the surface anionic sites; the reservoir of the bulk of intracellular (adsorbed) K^+ (which provides K^+ to the surface anionic sites thereby delaying the process of K^+ depletion in a low K^+ medium) has little effect on the ψ increase. Rather, the rapid decrease of ψ followed by *stepwise* reversal and hyperpolarization (Figure 10) suggests significant conformation changes of the cell surface proteins, reminiscent of the drastic electrical potential change of sea urchin and toad eggs

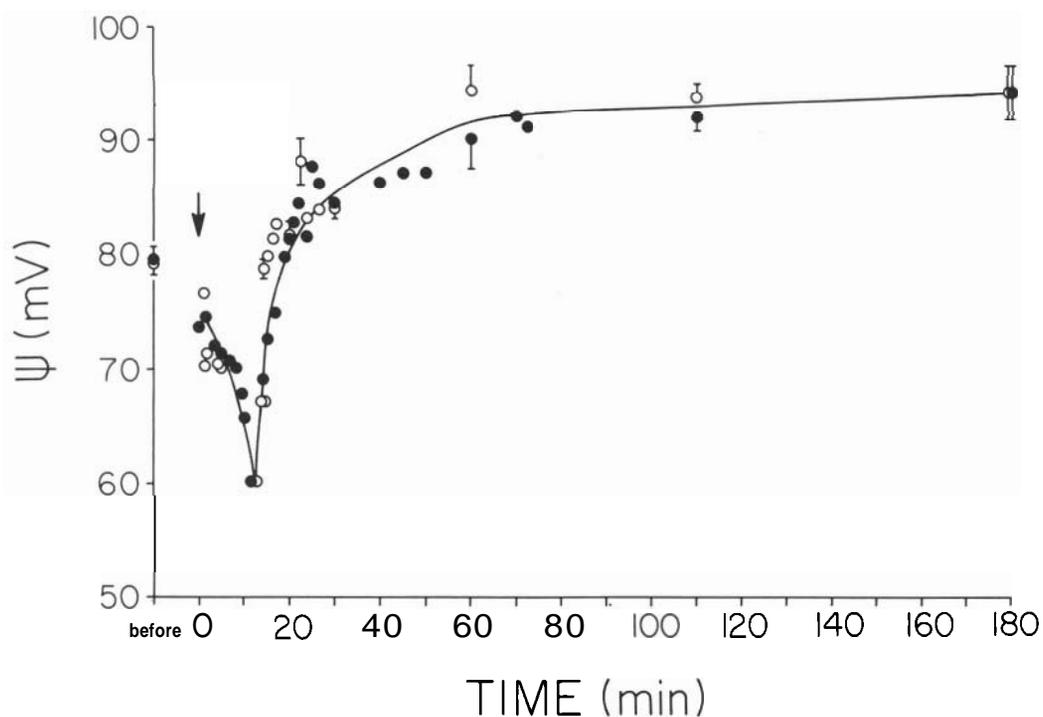


FIGURE 10. Time course of the change of the resting potential of frog sartorius muscles in 731 Ringer solution containing adrenalin (2.73×10^{-5} M) (25°C). Adrenaline was added at 0 time indicated by the arrow. Two sets of similar data combined.

following fertilization (Maeno, 1959; Shen and Steinhardt, 1978).

Variation of ψ with Different $[K^+]_{ex}$ in the Presence of Adrenaline. Figure 11 shows that exposure to adrenaline (3.83×10^{-5} M) raised ψ throughout the entire range of $[K^+]_{ex}$ studied. The curve that fits the ψ measured in adrenaline-treated muscle was calculated from Equation 4 with K_{Na-K}^{00} equal to 66.7 and $8 = 0.0736$ ($-\gamma/2 = 0.75$ Kcal/mole) in comparison with the control curve: $K_{Na-K}^{00} = 40$, $8 = 0.0736$ ($-\gamma/2 = 0.75$ Kcal/mole). Thus the most noticeable effect of 3.83×10^{-5} M adrenaline on ψ was to raise K_{Na-K}^{00} by a factor of $66.7/40 = 1.67$. However, if one returns to the theoretical curves shown in Figure 1 to Figure 4, one would notice that the theoretical ψ values with different K_{Na-K}^{00} (i.e., different ϕ) converge at high $[K^+]_{ex}$. The theoretically calculated best fitting curves of adrenaline-treated and control muscle, on the other hand, are separated by a more or less constant difference of several millivolts. This phenomenon recalls the effect of high concentration of Ca^{++} on the resting potential of frog muscle: a uniform elevation of ψ at all $[K^+]_{ex}$ over the range from normal (i.e., 2.5 mM) to as high as 100 mM, with no significant alteration of the slope of the ψ vs. $\ln [K^+]_{ex}$ (Ling and Gerard, 1949b; Jenerick and Gerard, 1953).

The Combined Effects of Adrenaline and High Ca^{++} on ψ at Different $[K^+]_{ex}$. The adrenaline data shown in Figure 11 were obtained by exposing frog sartorius muscle to normal 731 Ringer solution containing 3.83×10^{-5} M adrenaline and 1 mM Ca^{w} . A similar set of data is displayed in Figure 12 side by side with another set obtained from muscles treated with 731 Ringer solution containing the same concentration of adrenaline (3.83×10^{-5} M) but 10 mM Ca^{w} . The theoretical curve that fits the data for muscles exposed to adrenaline and 10 mM Ca^{++} was

calculated with K_{Na-K}^{00} of 66.7 and $8 = .0736$ ($-\gamma/2 = 0.75$ Kcal/mole). These values are identical to those describing the data from muscles exposed to adrenaline and 1 mM Ca^{++} . Yet, clearly these curves are not the same since one is higher than the other

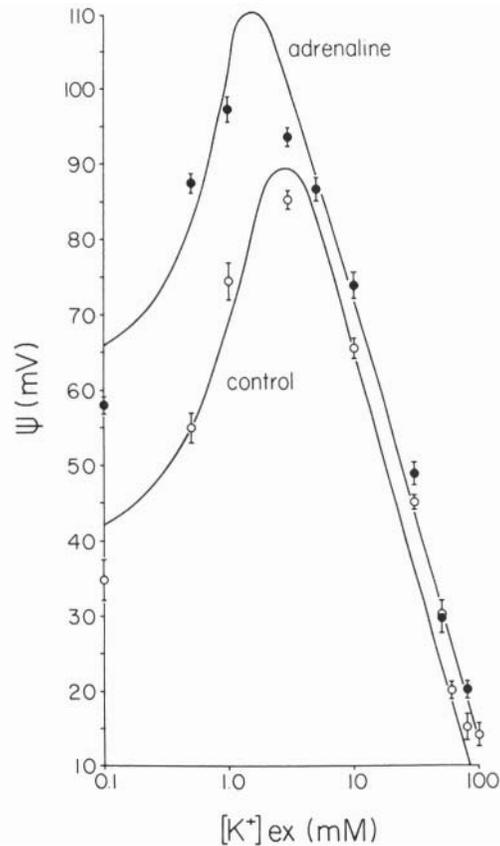


FIGURE 11. Resting potentials of frog sartorius muscles exposed to adrenaline (2.73×10^{-5} M) (25°C) and their controls. Muscles exposed to K^+ concentration equal to or higher than 2.5 mM, were exposed to Ringer solutions containing the different K^+ concentration and 2.73×10^{-5} M adrenaline for 2 hours (25°C). Muscles exposed to K^+ concentration lower than 2.5 mM, were incubated in several changes of the Ringer solution containing the right K^+ concentration and adrenaline for 3 days (25°C). Control muscles were treated the same way except all solutions contained no adrenaline. Solid lines were theoretical calculated according to Equation (4). For the adrenaline-treated muscle, $-\gamma/2$ was 0.75 Kcal/mole ($8 = 0.0736$), $K_{Na-K}^{00} = 66.7$ and constant₁ = 125 mV; for the control $-\gamma/2 = 0.75$ Kcal/mole ($\theta = 0.0736$), $K_{Na-K}^{00} = 40$, and constant₁ = 105 mV.

through the curve by about 12 mV. That is to say, the "constant," term on the right hand side of Equation 6 is higher by 12 mV in muscles exposed to the higher Ca^{++} concentration. As mentioned earlier, this "constant" includes implicitly the concentration of fixed anionic sites at the cell surface. How the

density of surface anionic sites can be increased by Ca^{++} will be discussed below.

DISCUSSION

The main experimental findings of this paper are that the effects of ouabain, adrenaline and Ca^{++} on the resting potential of frog muscle can be quantitatively described by Equation 4 based on the SA theory of the cellular electrical potential. Next we shall demonstrate that the ability of Equation 4 to describe quantitatively the resting potential of living cells is not limited to frog muscle, or to data obtained from this laboratory. In fact, it describes those very excellent experimental data which at one time had offered the best evidence in support of the electrogenic pump theory, a theory that has now been shown to be no longer tenable.

Depolarization by Ouabain and Hyperpolarization by Adrenaline and Ca^{++} . We shall begin with the observed effect of ouabain on the resting potential of isolated soleus muscle of rats fed a low K^+ diet reported by Akaike (1975). The author showed that in the presence of ouabain (10^{-4} M), the resting potential can be described by a modified Hodgkin-Katz equation in which the Cl^- terms have been dropped. Yet in the absence of ouabain the ψ vs. $[\text{K}^+]_{\text{ex}}$ curve can no longer be fitted with the same modified Hodgkin-Katz equation. In agreement with the earlier view of Marmor and Gorman, Akaike sought to explain this "hyperpolarization" in terms of an electrogenic pump which ouabain suppresses.

Figure 13 shows that Akaike's data on the resting potential of normal- and ouabain-treated soleus muscle can be described by Equation 4 with $K_{\text{Na-K}}^{\text{ou}}$ equal to 96.3 and 35 respectively. However, $-\gamma/2$, which only dipped from 1.0 to 0.56 Kcal/mole in the case of frog muscle (Figure 9); has dropped from 0.67 Kcal/mole to zero. This is to be expected

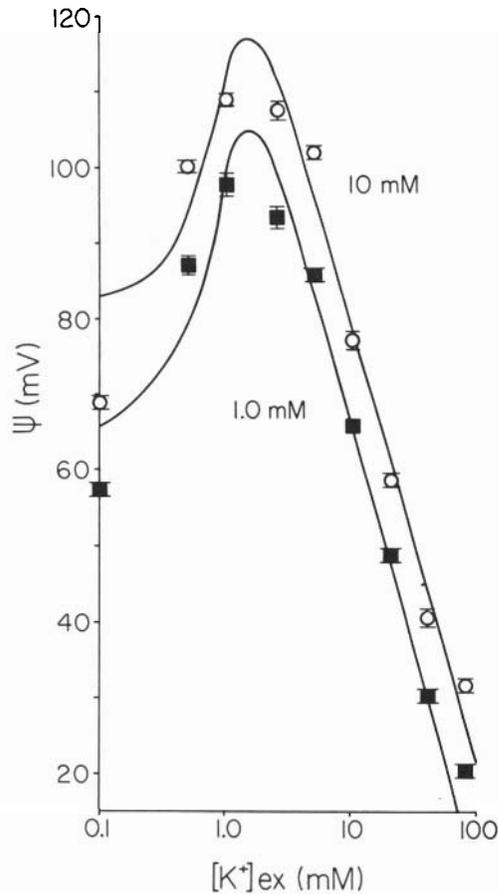


FIGURE 12. Effect of different concentrations of Ca^{++} and the same concentration of adrenaline (2.73×10^{-5}) on the resting potentials of frog sartorius muscle in Ringer solution containing different concentrations of K^+ . Experimental conditions were similar to those described under Figure 11; except in one set of data, the Ca^{++} concentration was 1.0 mM while in the other set it was 10 times higher. Solid lines were theoretical calculated according to Equation (4). For muscles treated with adrenaline + 1 mM Ca: $-\gamma/2 = 0.75$ Kcal/mole ($\delta = 0.0736$), $K_{\text{Na-K}}^{\text{ou}} = 66.7$, constant, = 120 mV; for muscles treated with adrenaline + 10 mM Ca: $-\gamma/2 = 0.75$ Kcal/mole ($\delta = 0.0736$), $K_{\text{Na-K}}^{\text{ou}} = 66.7$, and constant, = 131 mV.

since Akaike used ouabain at a concentration of 10^{-4} M and thus 133 times higher than we used (3.26×10^{-7} M). Previous studies showed that the bulk phase distribution of K^+ and Na^+ in guinea pig taenia coli obeyed Equation 1 with decline of $-\gamma/2$ (and K_{Na-K}^{00}) from 0.53 Kcal/mole (at ouabain concentration at 10^{-7} M) to 0 Kcal/mole (at ouabain concentration of 10^{-6} M) (Gulati, 1973).

One recalls that in the ouabain-treated frog muscles and their "controls" after reversal, the ψ measured in the presence of very high

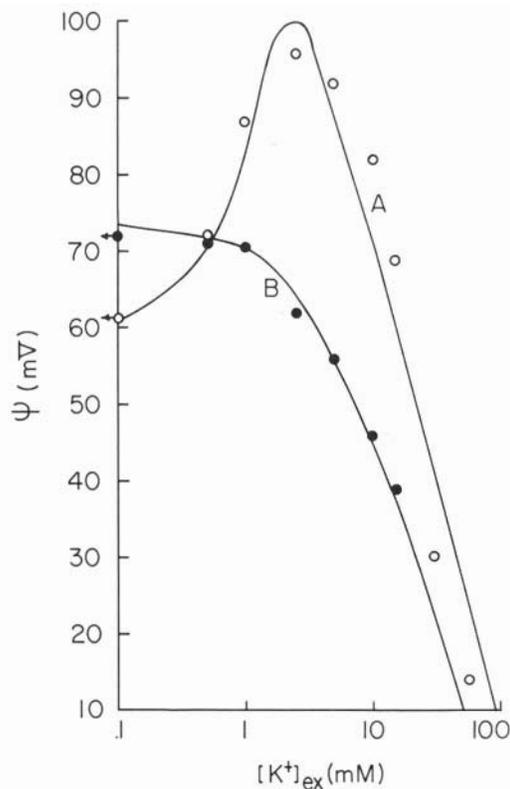


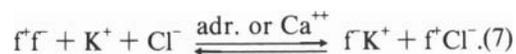
FIGURE 13. Effect of ouabain (10^{-4} M) on the resting potential of rat soleus muscles. A Na^+ -loaded soleus muscle and B, Na^+ -loaded muscles exposed to ouabain (10^{-4}). Data of Akaike (1975). Na^+ -loaded muscles isolated from rats fed on a low K^+ diet for 40 to 49 days was exposed to the various solutions containing different concentrations of K^+ for 1.5 min. or longer. Lines are theoretical calculated according to Equation (4). A: $-\gamma/2 = 0.67$ Kcal/mole ($\theta = 0.1$), $K_{Na-K}^{00} = 96.3$, constant₁ = 116 mV; B: $-\gamma/2 = 0$ Kcal/mole ($\theta = 1$), $K_{Na-K}^{00} = 35$, constant₁ = 76.4 mV.

external K^+ concentration (100 mM) was higher than that of the theoretical curves (Figure 9). No such departure was seen in the data of Akaike (Figure 13). However, the data of Figure 8 were obtained after *prolonged* exposure of the frog muscles to the *high* external K^+ concentration, while Akaike measured the ψ of his rat muscle fibers only 1.5 minutes after exposure to each new solution. To explain why the duration of exposure to high K^+ concentration affects ψ , we shall begin by tackling another observation earlier discussed: i.e., exposure of frog muscle to high calcium concentration and adrenaline, alone or together, raised ψ in such a way that the best fitting theoretical curve involves an increase in the "constant₁" of Equation 4.

As mentioned under Theory, this constant₁ of Equation 4 includes the concentration of surface fixed anionic sites, $[f^-]$. The need for an increase of this constant in order to fit the experimental data thus suggests that adrenaline and high concentration of Ca^{++} increase the $[f^-]$. But then how can the concentration of surface fixed anionic sites be increased?

According to the AI hypothesis, the increase and decrease of $[f^-]$ at the cell surface and within the cell are the molecular events underlying many physiological activities. Aside from K^+ , Na^+ , and other free cations, the fixed anionic sites (β - and γ -carboxyl groups) always have a potential alternative partner in the form of fixed cationic groups $[f^+]$ comprising the ϵ -amino groups and guanidyl groups of the same or other proteins. The coupling of a fixed anion to a fixed cation forms what Speakman and Hirst (1933) called a salt linkage (f^+f^-). (For recent in vitro confirmation of this concept see Ling and Zhang, 1984).

Thus we may tentatively suggest that adrenaline and Ca^{++} drive to the right the following reaction at the cell surface:



The liberated f adsorbs K^+ and increases ψ .

Reaction 7 may also be driven to the right without the intervention of the cardinal adsorbents Ca^{++} or adrenaline, if one merely increases the concentration of external K^+ . However, this reaction is cooperative in nature involving a long lag period. The slow release of f^- at the cell surface in response to high external K^+ concentration may then account for the "hyperpolarization" observed in muscles incubated for a long time in solutions containing high external K^+ concentration (with or without ouabain) (Figure 9) but not in muscles that had been exposed to the high external K^+ concentration only briefly (Figures 8, 12, 13).

Hyperpolarization by Valinomycin. Valinomycin, a highly toxic and therefore "useless" antibiotic produced by streptomyces fulvissimus, gained wide interest due to its demonstrated ability to increase dramatically the conductance of lipid membranes to K^+ but not to Na^+ (Andreoli et al., 1967). At 10^{-7} M, valinomycin was shown also to affect the resting potential of isolated mitochondria (Maloff et al., 1978). As a result, ψ became more negative by about 30 mV at $[K^+]_{ex}$ of 10 mM. However, valinomycin did not change the K^+ permeability or conductance of the liver mitochondria membrane as expected if valinomycin acts as a specific K^+ carrier or ionophore through a lipid membrane.

It has been shown that the experimental data of Maloff et al. can also be explained with the aid of Equation (4) (Ling, 1982) with the assumption that 10^{-7} M valinomycin increases the intrinsic equilibrium constant (K_{x-k}^{oo}) of the mitochondrial surface anionic sites by a factor of 3.0 and constant, by 30 mV as shown in Figure 14. The nature of the alternative adsorbent x is unspecified. It might be H^+ . The required increase of constant₁ suggests valinomycin drives the reaction at the cell surface described by Equation (7) to the right.

Hyperpolarization by Lowering External Ca^{++} Concentration. Next we analyze yet another set of published data in the literature, i.e., the data of Bruce and Anderson (1979) on the hyperpolarization of mouse parathyroid cells by low Ca^{++} concentration. The theoretical curves shown in Figure 15 based on Equation (4) that fit most of the data points were calculated with K_{Na-k}^{oo} equal to 2.7 and $\theta = 0.4$ ($-\gamma/2 = +0.27$ Kcal/mole) for parathyroid cells exposed to 2.5 mM Ca^{++} and K_{Na-k}^{oo} equal to 19.7 and $\theta = 0.3$ ($-\gamma/2 = 0.35$ Kcal/mole) for parathyroid cells exposed to the lower Ca^{++} concentration of 1.5

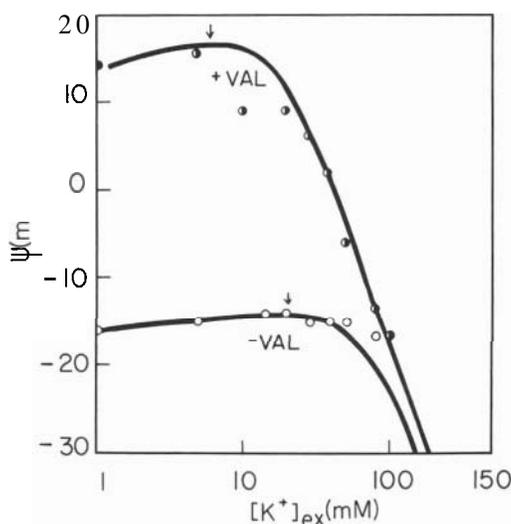


FIGURE 14. Effect of valinomycin (10^{-7} M) on the resting potential of giant liver mitochondria from mouse fed cuprizone. Data points are from Maloff et al. (1978). Lines are theoretical according to Equation (4). For the control (- VAL): $-\gamma/2 = 0.2$ Kcal/mole ($0 = 0.5$), constant₁ = 0.8 mV; for the mitochondria treated with valinomycin (+ VAL): $-\gamma/2 = 0.2$ Kcal/mole ($0 = 0.1$), constant₁ = 31 mV. Intrinsic equilibrium constants K_{x-k}^{oo} are not given since the alternative counterion (x) to K^+ and its concentrations are not known. However, apparent equilibrium constants (K_K^{oo}) in (M^{-1}) are given where $K_K^{oo} = K_{x-k}^{oo} / [x]_{ex}$ and $[x]_{ex}$ is the molar concentration of x . For mitochondria without valinomycin, K_K^{oo} equals 50 (M^{-1}); for mitochondria treated with valinomycin, K_K^{oo} equals 16.6 (M^{-1}). (Figure from Ling, 1981, except the convention of the polarity of the potential was the opposite of one shown here, i.e., ψ is positive when the inside of the cell is negative to the external medium.)

mM. Thus a change of Ca^{++} concentration in the external medium from 2.5 mM to 1.5 mM, raised $K_{\text{Na-K}}^{\circ}$ 7.3 fold from 2.8 to 20.

That increase of Ca^{++} depresses ψ in parathyroid cells but increases ψ in frog muscle is one of the apparently contradictory effects often seen in physiological reactions. Possible molecular mechanisms have been discussed elsewhere. Briefly, it has been shown that the exposure to one cardinal adsorbent may produce opposite physiological effects depending on whether or not a second cardinal adsorbent is present (Ling, 1962, 1981, 1984, p. 204). How hyperpolarization of the parathyroid cells produced by a reduction of external Ca^{++} concentration leads to the liberation of parathyroid hormone and mobilization of Ca^{++} is not exactly understood. It is interesting that more recently

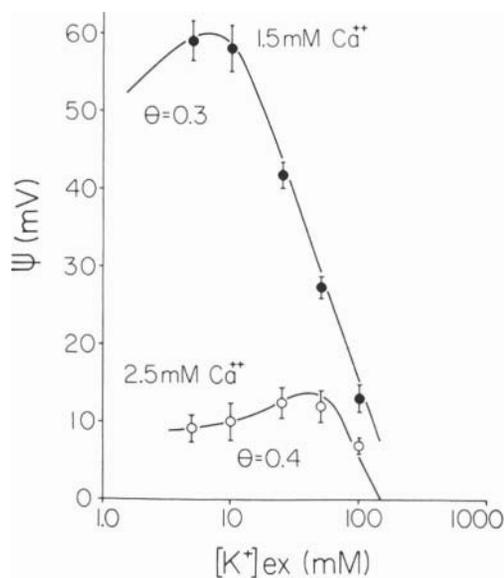


FIGURE 15. Effect of low concentration of external Ca^{++} on the resting potential of mouse parathyroid cells. Data points are those of Bruce and Anderson (1979). The lines are theoretical calculated from Equation (4). For cells in 2.5 mM Ca^{++} , $-\gamma/2 = 0.27$ Kcal/mole ($\theta = 0.4$), $K_{\text{Na-K}}^{\circ} = 2.7$ and constant, = 30 mV. For cells in 1.5 mM Ca^{++} , $-\gamma/2 = 0.35$ Kcal/mole ($\theta = 0.3$), $K_{\text{Na-K}}^{\circ} = 19.7$, and constant₁ = 76.8 mV.

Souhrada and Souhrada (1984) observed hyperpolarization of airway smooth muscle cells of guinea pig in response to active and passive immunological sensitization. This immunologically-induced hyperpolarization is accompanied by contraction of the smooth muscle, a response believed to underlie the bronchial asthma produced. These authors explained their findings on the basis of the electrogenic Na pump. Unfortunately their data are not complete enough to lend themselves to the kind of analysis described here.

Alternative Interpretations of Phenomena so Far Explained Only on the Basis of the Electrogenic Pump. In the following, we shall review briefly experimental observations which have become widely regarded as specific supportive evidence for the electrogenic pump theory and attempt to demonstrate that most, if not all, can be explained by the surface adsorption theory with a minimum of additional ad hoc postulations.

(1) K^+ ACTIVATED HYPERPOLARIZATION (KAH). Cells loaded with Na^+ and depleted of K^+ by exposure to a K^+ -free Ringer solution, were returned to a medium containing the normal concentration of K^+ ψ returned to its initial normal value long before intracellular K^+ concentration returned to its normal level (Kernan, 1962; Cross et al., 1965; Adrian and Slayman, 1966; Page and Storm, 1965; Akaike, 1975). This disagreement between ψ measured and that predicted by the intracellular K^+ concentration contradicts the membrane theory of the cell electrical potential which predicts that ψ directly depends on $\ln [\text{K}^+]_{\text{in}}$. This dissociation of ψ and $[\text{K}^+]_{\text{in}}$ on the other hand, is in accord with the SA theory, according to which the ψ depends only on the concentration of the adsorbed K^+ at a microscopically thin layer of the cell surface and not on the K^+ concentration in the bulk phase cytoplasm. The rapid recovery of ψ parallels the rapid restor-

ation of K^+ concentration of the cell surface (Ling and Fisher, 1983, Figure 2).

(2) **DEPOLARIZATION BY OUABAIN AND METABOLIC POISONS.** Ouabain, once widely believed to be a specific inhibitor of the Na pump, causes the loss of K^+ and gain of Na^+ in intact muscle cells as well as muscle cell preparations without a functional cell membrane and postulated pumps (Ling, 1978). This experiment suggests that the action of ouabain is that of a typical cardinal adsorbent. It acts directly on the K^+ - Na^+ adsorbing proteins increasing the electron density or c -value of the β - and γ -carboxyl groups and bringing about a decrease of K_{Na-K}^{00} as well as $-\gamma/2$ as Figures 9 and 13 have shown. (For earlier evidence, see Ling and Bohr, 1971b.)

(3) **HYPERPOLARIZATION PRODUCED BY "INJECTION" OF Na^+ INTO SNAIL NEURONES.** Kerkut and Thomas (1965) demonstrated a marked hyperpolarization of snail neurones when Na chloride or acetate were allowed to diffuse out of the tip of a low-impedance glass capillary inserted inside the cell. This hyperpolarization effect was not produced by similar "injection" of the K^+ or Li^+ salts and was inhibited by ouabain, p-chloromercuribenzoate or a reduction of external K^+ . Based on these observations the authors concluded that the hyperpolarization produced by Na^+ "injection" was due to the stimulation of an electrogenic sodium pump by the high concentration of intracellular Na^+ .

We would like to suggest the following alternative interpretation of these interesting phenomena: The hyperpolarization produced by Na^+ "injection" is a transient one, lasting less than 10 minutes with each short burst of Na^+ salt "injection" (see Thomas, 1969). The time course of ψ change closely follows that of the intracellular Na^+ concentration, reaching a peak $\Delta\psi$ value at the time when $[Na^+]_i$ is at its peak, declining with dissipation of intracellular Na^+ . These characteristics bear re-

semblance to those of an ordinary diffusion potential. However, obviously it cannot be a diffusion potential of Na^+ and Cl^- (or acetate) in a simple dilute aqueous solution, in which case, the potential should be of opposite polarity since the mobility of Cl^- is faster than Na^+ in water. To explain the hyperpolarization by the infusion of Na^+ salt the requirement is that at the "bottleneck" of diffusion, Na^+ must have a higher mobility than K^+ , Li^+ , or Cl^- . We would like to suggest that these requirements may be met at the surface of the snail neurone cells which may contain a two-dimensional gridwork of fixed anionic sites strongly preferring K^+ over Na^+ similar to that seen in muscle cells. In that case, one may anticipate the following:

a. **LOWER Cl^- PERMEABILITY THAN THAT OF K^+ :** The anionic nature of the surface grid of fixed charges can be expected to repel and resist passage of anions like Cl^- more effectively than it resists the passage of cations like K^+ or Na^+ . In support of this view, we would like to mention that in studying the effect of external KCl concentration on the ψ of frog muscle cells one observes that ψ reversed its size when external KCl concentration reached 200 mM or higher (Ling, unpublished). This polarity reversal shows that the cell surface selectivity allows speedier passage of K^+ than Cl^- .

b. **HIGHER PERMEABILITY TO Na^+ THAN K^+ :** Under normal resting conditions the surface anionic sites of many cell surfaces strongly prefer K^+ over Na^+ . Thus would-be effluxing K^+ from inside the cell tends to be attracted to and (momentarily) captured by the surface anions. In contrast, the less preferred Na^+ has a higher probability of leaving the cell without interruption. This idea is substantiated by the much faster half time of exchange of labelled Na^+ (i.e., a few minutes) than that of labelled K^+ (i.e., 5 to 6 hours) in frog muscle (Ling, 1980, 1984, p. 404) and by the much

slower equilibration of K^+ than Na^+ in amphibian oocytes (Horowitz and Paine, 1979). From these considerations, one may anticipate that after the infusion of $NaCl$ or Na acetate into the neurones, a diffusion potential was set up at the snail neurone cell surface, where the Na^+ injected outstrips the Cl^- , giving rise to an extra 20 to 30 mV. It must be pointed out that the quantity of Na^+ involved in generating this hyperpolarization is so extremely small that it is not likely to be detectable chemically (see Ling, 1981; Guggenheim, 1950, p. 330). In addition, it should be noted that high ξ (Equation 2) creates the K^+ -loaded, Na^+ repelling surface anionic grids.

The suppression of hyperpolarization by ouabain, p-chlorimercurbenzoate and low external K^+ is caused by a reduction of ξ of the cell surface sites. This is a consequence of reducing K_{Na-K}^{00} , as in the case of ouabain (Figures 9 and 13), or by reducing $[K^+]_o$. When ξ is greatly reduced, Na^+ will also be captured by the surface anions and the condition for Na^+ moving ahead of the Cl^- disappears. As a result the hyperpolarization produced by the injected $NaCl$ vanishes with it.

C. WARMING-INDUCED HYPERPOLARIZATION: Another observation often cited in support of the electrogenic pump theory is the hyperpolarization produced by warming of certain types of cells including the molluscan neurones. The new interpretation offered is based on the concept of a "temperature transition" of the cooperatively linked surface ionic sites. This subject will be dealt with in a following paper (Ling, 1984c).

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