STUDIES ON ION DISTRIBUTION IN LIVING CELLS

II. Cooperative Interaction between Intracellular Potassium and Sodium Ions

GILBERT N. LING *and* GRACE BOHR

From the Department of Molecular Biology, Division of Neurology, Pennsylvania Hospital, Philadelphia, Pennsylvania 19107

ABSTRACT New steady levels of K^+ and Na^+ ion in frog sartorius muscle were reached in 72 hr at 25°C in environments containing 100-mM Na^+-ion and K^+-ion concentrations varying from near zero to 10 mM. These steady levels follow a pattern predicted by a cooperative adsorption isotherm presented in 1964. From a total of 13 sets of experiments carried out over a five year period, the average concentration of adsorption sites is 109 μmoles/g of fresh cells. The average intrinsic equilibrium constant for the Na → K exchange is 135, and the average free energy of nearest neighbor interaction is 0.54 kcal/mole. These values are in good agreement with those obtained by Jones and Karreman from studies on canine carotid arteries.

INTRODUCTION

According to the membrane theory, all cells are enclosed in lipoid-protein membranes with small pores. The steady level of each solute in the cell is maintained either by an absolute impermeability of the membrane to the solute or by a balance of various inward and outward non–energy-consuming transport mechanisms (free and facilitated diffusion) and inward and outward pumping actions (Pfeffer, 1921; Overton, 1907; Boyle and Conway, 1941; Dean, 1941; Danielli, 1954; Wilbrandt and Rosenberg, 1961; Cohen and Monod, 1957).

Though widely held, the membrane theory is not the only theory that has been proposed (Fischer and Moore, 1908; Ernst, 1963; Troschin, 1958; Ling, 1952). Thus, according to the association-induction hypothesis, intracellular solute may exist in two states: interstitial solute dissolved in the cell water, and adsorbed solute on fixed sites (Ling, 1962, 1964 *a*, 1965 *a*, 1969). The interstitial solute is, as a rule, found at a concentration lower than that in the surrounding medium because of a lower entropy of the solute in the cell water which exists as polarized multilayers on the cell proteins (Ling, 1965 *b*). Thus in normal resting frog muscle, about half of intracellular Na^+

Reprinted from the Biophysical Journal, 1970, Vol. 10, No. 6, pp. 519–538 *Printed in U.S.A.*

ion (*circa* 10 mM) is considered to be interstitial even though the Na^+-ion concentration in the surrounding medium is ten times higher. Evidence that the cell water exists in a quasicrystalline state has come from intracellular freezing experiments (Ling, 1967, 1968), and from nuclear magnetic resonance (NMR) studies (Chapman and McLauchlan, 1967; Cope, 1969; Hazlewood, Nichols, and Chamberlain, 1969; Fritz and Swift, 1967). Evidence that water in such a physical state partially excludes solutes like Na^+ ion (and sugars) for entropic reasons has come from equilibrium distribution studies in the water within model systems such as sheep's wool (Ling, 1962, 1965 *b*) and cellulose-acetate membranes.[1]

Adsorbed solutes may be low in concentration but may also reach concentrations considerably higher than that in the surrounding medium. Often this adsorption exhibits a high degree of specificity. The bulk of intracellular K^+ ion, according to the association-induction hypothesis, is adsorbed. Evidence for this comes from studies of the distribution of K^+, Rb^+, and Cs^+ ion in frog muscles at high concentrations (2.5 mM and higher): the equilibrium intracellular ion concentration in this case follows the Langmuir adsorption isotherm (Ling and Ochsenfeld, 1966). Other evidence that the bulk of intracellular K^+ ion exists in an adsorbed state has been obtained from NMR studies of Na^+ ion in K-depleted muscles. These studies show that the Na^+ ion which replaces the lost K^+ does not exhibit the sharp adsorption peak typical of free Na^+ ion and is adsorbed (Ling and Cope, 1969).

The association-induction hypothesis also contends that the adsorption sites for K^+ and Na^+ ion are the β- and γ-carboxyl group of the cell proteins (Ling, 1952; Ling, 1962; Ling and Ochsenfeld, 1966). When two species of ions with widely different adsorption energies are both present in the surrounding medium, the adsorption of these ions should no longer follow the simple Langmuir adsorption isotherm. Instead, cooperative interaction between nearest neighbor sites will be evident.

A cooperative adsorption isotherm was presented by Yang and Ling in 1964 (Ling, 1964 *b*)[2] and shown to apply to a wide variety of adsorption on proteins in vitro (Ling, 1966). Subsequently, Karreman gave the details of the derivation of this isotherm and went on to deal with its more complex variations (Karreman, 1965). Recently, Jones and Karreman published results of their experimental study on the K^+- and Na^+-ion distribution in canine carotid arteries, confirming the theory (Jones and Karreman, 1969 *a* and *b*). In the present report we shall present a complete account of our studies of K^+- and Na^+-ion distribution in frog sartorius muscle in a range of K^+-ion distribution from very low to above normal. A preliminary report of these findings was presented earlier (Ling, 1966: see also Ling, 1965 *c*).

---

[1] Ling, G. N., and S. Will. 1969. *Physiol. Chem. Phys.* In press.

[2] Since then, formal cooperative adsorption isotherms were derived by Monod, Wyman, and Changeux (1965) and by Koshalnd, Nemethy, and Filmer (1967). A comparison of our model with that of Monod et. al. has been given elsewhere (Ling, 1969).

THEORY

The Model According to the Association-Induction Hypothesis

An adsorption isotherm was derived, using the familiar one-dimensional Ising model (see Ling, 1964 *b*; Karreman, 1965); we considered long protein chains possessing similar sites, which can adsorb either K^+ or Na^+ ion as described by the following equations:

$$[K^+]_{ad} = \frac{[f]}{2}\left[1 + \frac{\xi - 1}{\sqrt{(\xi - 1)^2 + 4\xi \exp(\gamma/RT)}}\right], \quad (1)$$

and

$$[Na^+]_{ad} = \frac{[f]}{2}\left[1 - \frac{\xi - 1}{\sqrt{(\xi - 1)^2 + 4\xi \exp(\gamma/RT)}}\right], \quad (2)$$

where R and T are the gas constants and absolute temperature, respectively, an $[f]$ represents the concentration of adsorption sites. ξ is defined as follows:

$$\xi = K^{00}_{Na\to K}\frac{[K^+]_{ex}}{[Na^+]_{ex}}, \quad (3)$$

and

$$\Delta F^{00}_{Na\to K} = -RT \ln K^{00}_{Na\to K}, \quad (4)$$

where $K^{00}_{Na\to K}$ is the *intrinsic equilibrium constant* for the K^+-ion and Na^+-ion exchange and $\Delta F^{00}_{Na\to K}$ is the corresponding *intrinsic free energy* change when an adsorbed Na^+ ion is replaced by a K^+ ion, without, at the same time, creating new $K^+ \cdot Na^+$ neighboring pairs. $-\gamma/2$ represents the *free energy of nearest neighbor interaction.*

If three neighboring sites are all originally adsorbing Na^+ ion, and if the middle site now exchanges its Na^+ ion for a K^+ ion, the free energy change is not merely $\Delta F^{00}_{Na\to K}$ but is $[\Delta F^{00}_{Na\to K} + 2(-\gamma/2)]$ or $(\Delta F^{00}_{Na\to K} - \gamma)$, because in this exchange, $Na \cdot Na \cdot Na \rightarrow Na \cdot K \cdot Na$, two new $K \cdot Na$ neighboring pairs are created, and each pair entails an additional amount of free energy equal to $-\gamma/2$. On the other hand, in an exchange of the middle Na^+ ion for a K^+ ion in a triad of sites $K \cdot Na \cdot Na$ (to $K \cdot K \cdot Na$), the free energy change is simply $\Delta F^{00}_{Na\to K}$, since no net gain or loss in the number of $K \cdot Na$ neighboring pairs has resulted.

Dividing equation 1 by equation 2, one obtains the ratio for the concentration of adsorbed K^+ and Na^+ ions:

$$\frac{[K^+]_{ad}}{[Na^+]_{ad}} = \frac{\sqrt{(\xi - 1)^2 + 4\xi \exp(\gamma/RT)} + \xi - 1}{\sqrt{(\xi - 1)^2 + 4\xi \exp(\gamma/RT)} - \xi + 1}. \quad (5)$$

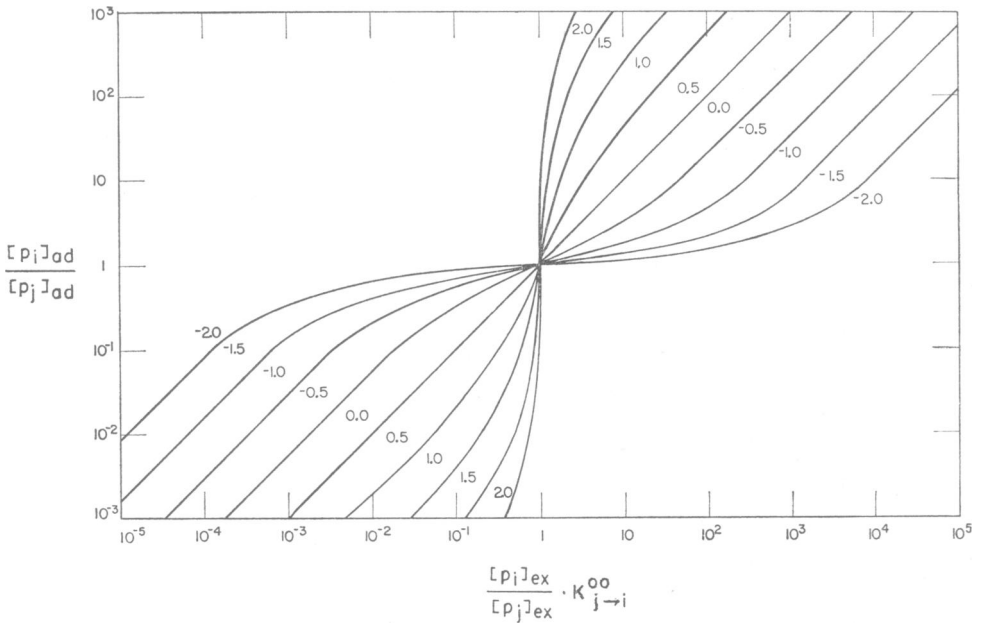

FIGURE 1 Theoretical plot of the cooperative adsorption of two solutes. Theoretical curves are calculated according to a generalized version of equation 5, where $[K^+]_{ad}$ and $[Na^+]_{ad}$ are replaced by $[p_i]_{ad}$ and $[p_j]_{ad}$, and $[K^+]_{ex}$ and $[Na^+]_{ex}$ by $[p_i]_{ex}$ and $[p_j]_{ex}$, respectively. The numbers near each curve refer to the free energy of nearest neighbor interaction ($-\gamma/2$) and are in kcal/mole, calculated for a temperature of 25°C. The abscissa represents normalized values of ($[p_i]_{ex}/[p_j]_{ex}$) by multiplying it with $K^{oo}_{j \to i}$.

In Fig. 1, $\log([K^+]_{ad}/[Na^+]_{ad})$ is plotted against $\log([K^+]_{ex}/[Na^+]_{ex})$ according to equation 5. In these plots, as $([K^+]_{ex}/[Na^+]_{ex})$ becomes very large or very small, the slope of the curve approaches unity. In the middle range of the curves, the slope varies with $-\gamma/2$. Thus, the tangent to any one of the curves at the locus $([K^+]_{ad}/[Na^+]_{ad}) = 1$ (where each ion occupies one-half of the available sites) may be described by an equation of the form

$$\log\left(\frac{[K^+]_{ad}}{[Na^+]_{ad}}\right) = n \log\left(\frac{[K^+]_{ex}}{[Na^+]_{ex}}\right) + n \log K. \qquad (6)$$

Equation 6 is, in fact, formally identical with a familiar empirical equation, that of A. V. Hill (Hill, 1910), for the binding of oxygen on hemoglobin (discussed below in more detail). By setting $([K^+]_{ad}/[Na^+]_{ad}) = 1$ and differentiating equation 5, one finds that $K = K^{oo}_{Na \to K}$ and that n is explicitly related to $-\gamma/2$ as follows (see Ling, 1964 *b*):

$$n = \exp\left(-\frac{\gamma}{2RT}\right). \qquad (7)$$

When $n > 1$, $-\gamma/2 > 0$, it is energetically more favorable for two neighboring sites to adsorb the same ion; the adsorption of K^+ and Na^+ ion on neighboring sites is less favorable. This type of cooperative adsorption is referred to as *autocooperative* adsorption (Ling, 1962, 1964 *b*, 1966). On the other hand, when $n < 1$, $-\gamma/2 < 0$, it is energetically more favorable to have K^+ and Na^+ ion adsorbed on neighboring sites. This type of cooperative adsorption is referred to as *heterocooperative* adsorption. When $n = 1$, $-\gamma/2 = 0$, equation 1 simplifies into:

$$[K^+]_{ad} = \frac{[f][K^+]_{ex}}{\tilde{K}^{00}_{Na\to K}[Na^+]_{ex} + [K^+]_{ex}}.$$

This, of course, is the Langmuir adsorption isotherm when the number of vacant sites is negligible.

General Equation for Solute Distribution in Living Cells

The general equation for the equilibrium distribution of solutes, when applied to the K^+ ion in a living cell in the presence of Na^+ ion, is as follows (Ling, 1965 *c*, 1966):

$$[K^+]_{in} = \alpha q_K [K^+]_{ex} + \sum_{L=1}^{N} \frac{[f]_L}{2}\left[1 + \frac{\xi^L - 1}{\sqrt{(\xi^L - 1)^2 + 4\xi^L \exp(\gamma^L/RT)}}\right], \quad (9)$$

where $[K^+]_{in}$ is the total intracellular K^+-ion concentration, α is the percentage of water in the cell, and q_K is the equilibrium distribution coefficient of K^+ ion between the cell water and the external medium. $[f]_L$ is the concentration of the Lth type of adsorption sites among a total of N types. ξ^L and γ^L are as defined above in reference to the Lth type of sites. In the simple case when there is only one type of adsorption site, equation 9 simplifies to

$$[K^+]_{in} = \alpha q_K [K^+]_{ex} + \frac{[f]}{2}\left[1 + \frac{\xi - 1}{\sqrt{(\xi - 1)^2 + 4\xi \exp(\gamma/RT)}}\right]. \quad (10)$$

The corresponding equation for Na^+ ion is

$$[Na^+]_{in} = \alpha q_{Na} [Na^+]_{ex} + \frac{[f]}{2}\left[1 - \frac{\xi - 1}{\sqrt{(\xi - 1)^2 + 4\xi \exp(\gamma/RT)}}\right], \quad (11)$$

where q_{Na} is the equilibrium distribution coefficient of Na^+ ion between cell water and the external medium.

Membrane-Pump Model

The membrane theory has not yet presented a general equation to describe the steady-level distribution of solutes in the living cell. Neither is there a specific quantitative formulation for the Na-pump theory. However, Wilbrandt, Widdas, and their coworkers presented an equation of the following form for the steady state dis-

tribution of glucose between the cell and its environment (Wilbrandt and Rosenberg, 1961):

$$\frac{V_{max}[S]_{in}}{K_{in} + [S]_{in}} = \frac{V'_{max}[S]_{ex}}{K'_{ex} + [S]_{ex}}, \tag{12}$$

where $[S]_{in}$ and $[S]_{ex}$ refer to the steady-state concentration of glucose in the cell water and in the external solution, respectively, V_{max} is the maximum outward rate of transport, and K_{in} the dissociation constant of the glucose-outward carrier complex. V'_{max} and K'_{ex} are the corresponding terms for the inward transport.

Another equation was introduced by Cohen and Monod (1957). In this case the steady-level sugar accumulation in bacteria was postulated to represent a "permease"-catalyzed entry of the sugar balanced against an exit, independent of permeases or carriers. Representing sugar concentration in the cell as $[S]_{in}$, they suggested that

$$\frac{g[S]_{ex}}{K + [S]_{ex}} = c[S]_{in}, \tag{13}$$

where g is the permease activity, K is the dissociation constant of the sugar-permease complex, and c the exit constant.

In all these cases, the carrier or pump is assumed to combine reversibly with sugars according to a Langmuir adsorption isotherm. A plot of the intracellular sugar concentration against the extracellular sugar concentration is typically a hyperbola, or resembles a hyperbola. The increment of $[S]_{in}$, for example, per unit of increment of $[S]_{ex}$ is higher at a low $[S]_{ex}$, becoming smaller as $[S]_{ex}$ increases.

Method of Data Analysis

For the sake of general applicability, we shall represent the two alternative species of adsorbents as p_i and p_j. (In our present study p_i and p_j may stand for K^+ and Na^+, respectively). $[p_i]_{ex}$, $[p_j]_{ex}$, $[p_i]_{ad}$, $[p_j]_{ad}$ represent the concentrations of external and adsorbed ith or jth solute. From a plot of $\log([p_i]_{ad}/[p_j]_{ad})$ against $\log([p_i]_{ex}/[p_j]_{ex})$ (see Fig. 1), one can estimate the values of $K^{00}_{j\to i}$ and $-\gamma/2$. To obtain the best-fitting theoretical curve for a set of experimental points, one begins by fitting a straight line to the points in the vicinity of $([p_i]_{ad}/[p_j]_{ad}) = 1$. The reciprocal of $([p_i]_{ex}/[p_j]_{ex})$ at $([p_i]_{ad}/[p_j]_{ad}) = 1$ is $K^{00}_{j\to i}$. The slope of this straight line is, as shown in equation 7, equal to n. Let us define a parameter θ, such that

$$\theta = \exp(\gamma/RT). \tag{14}$$

From equations 7 and 14, we have

$$\theta = \frac{1}{n^2}. \tag{15}$$

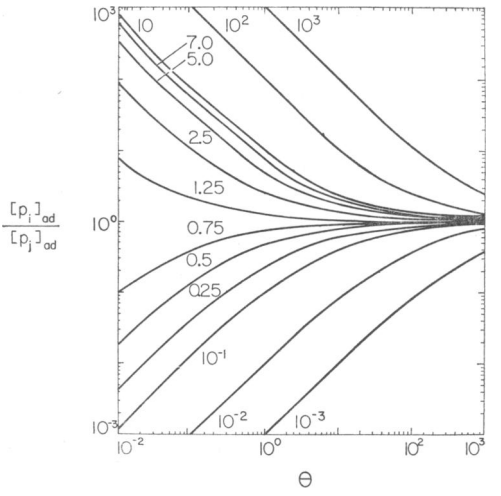

FIGURE 2 Theoretical plot of $([p_i]_{ad}/[p_j]_{ad})$ against θ. Theoretical curves are calculated according to a generalized version of equation 5, where $[K^+]_{ex}$ and $[K^+]_{ad}$ are replaced by $[p_i]_{ex}$ and $[p_i]_{ad}$ and $[Na^+]_{ex}$ and $[Na^+]_{ad}$ by $[p_j]_{ex}$, and $[p_j]_{ad}$, respectively. $\theta = 1/n^2 = \gamma/RT$. Numbers on the curves are the values of $([p_i]_{ex}/[p_j]_{ex})$.

Fig. 2 represents a different plot of equation 5. In contrast to Fig. 1, $([p_i]_{ad}/[p_j]_{ad})$ is plotted here against θ rather than $([p_i]_{ex}/[p_j]_{ex})$. By calculating the value of θ from the value of n obtained from the experimental data, one can read off, from the curves in Fig. 2, the theoretical value of $([p_i]_{ad}/[p_j]_{ad})$ [corresponding to each value of $([p_i]_{ex}/[p_j]_{ex})$] which are then plotted on a log-log scale against $([p_i]_{ex}/[p_j]_{ex})$ to obtain the *theoretical* curves for specific adsorption data.

MATERIALS AND METHODS

All the experiments presented here were performed on isolated sartorius muscles of fed leopard frogs (*Rana pipiens pipiens*, Schreber).

All materials and methods used were those given in the preceding paper of this series. Sterile incubation media containing different K^+-ion concentration (0.05–10 mM) but a constant concentration of Na^+ ion (100 mM) were prepared by mixing in different proportions the two stock solutions containing respectively 0.05 mM ("ACDE" solution) and 10 mM K^+ ion ("BCDE" solution). The composition of these solutions and their method of preparations, as well as the procedure of sterile tissue dissection and incubation, ion extraction and analysis, were also the same as described earlier (Ling and Bohr, 1969).

The formulas for calculating the total intracellular K^+- and Na^+-ion concentrations, $[K^+]_{in}$ and $[Na^+]_{in}$ are as follows:

$$[Na^+]_{in} = \frac{[Na^+]_{tis} - 8.5}{0.9}, \qquad (16)$$

and

$$[K^+]_{in} = \frac{[K^+]_{tis} - 0.54\,[K^+]_{ex}}{0.9}, \qquad (17)$$

for $[K^+]_{ex}$ under 2 mM; and

$$[K^+]_{in} = \frac{[K^+]_{tis} - 0.13\,[K^+]_{ex} - 0.65}{0.9}, \qquad (18)$$

for $[K^+]_{ex}$ between 2 and 10 mM. $[Na^+]_{tis}$ and $[K^+]_{tis}$ are the total tissue concentrations of Na^+ and K^+ ions, respectively. They, as well as $[K^+]_{in}$ and $[Na^+]_{in}$, are in millimoles per gram of tissue or cells as the case may be. The formulas for calculating the concentration of intracellular adsorbed K^+ ion, $[K^+]_{ad}$, and of adsorbed intracellular Na^+ ion, $[Na^+]_{ad}$, are, respectively,

$$[K^+]_{ad} = [K^+]_{in} - 0.1\,[K^+]_{ex}, \qquad (19)$$

and

$$[Na^+]_{ad} = [Na^+]_{in} - 0.1\,[Na^+]_{ex}. \qquad (20)$$

These formulas give the corrections for the extracellular space, the connective tissue, and the ion in the intracellular water. Their experimental basis and derivation are given in the Appendix to this article.

RESULTS

The Length of Time to Reach a New Ionic Equilibrium

When sartorius muscles were introduced into a medium containing a fixed concentration of Na^+ ion (100 mM) but different K^+-ion concentrations, the time course to reach a new steady level varied with the K^+-ion concentration. When the external K^+-ion concentration is higher than that in a normal Ringer solution, new steady levels are reached within 24 hr (Fig. 3), as has been shown earlier (Ling and Ochsenfeld, 1966). The time needed to reach a new steady level is much longer when the

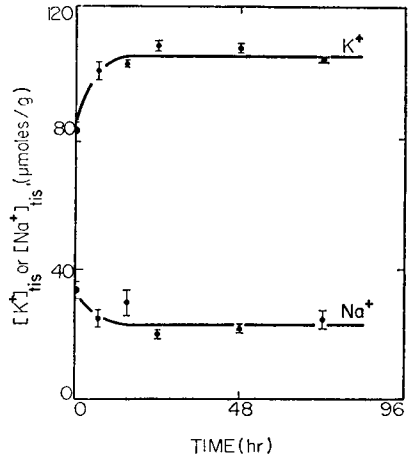

FIGURE 3 Time course of K^+-ion and Na^+-ion content changes in frog muscles in a medium containing an above normal K^+-ion concentration. The K^+-ion and Na^+-ion contents of the medium were initially 2.5 mM and 100 mM, respectively.

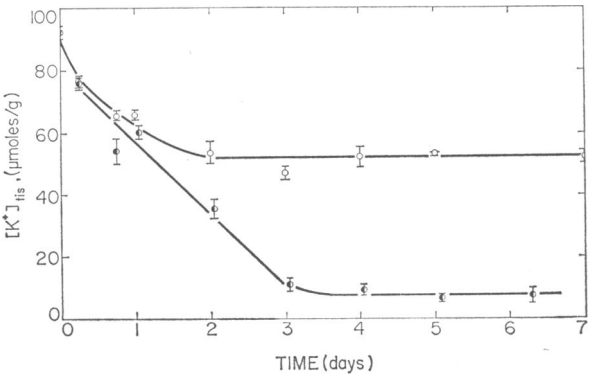

FIGURE 4 Time course of K^+-ion content changes in frog muscles in media containing below normal K^+-ion concentrations. Media contained 100 mM Na^+-ion in both sets of data, but different K^+-ion concentration (0.1 mM [◐] and 0.7 mM [○]).

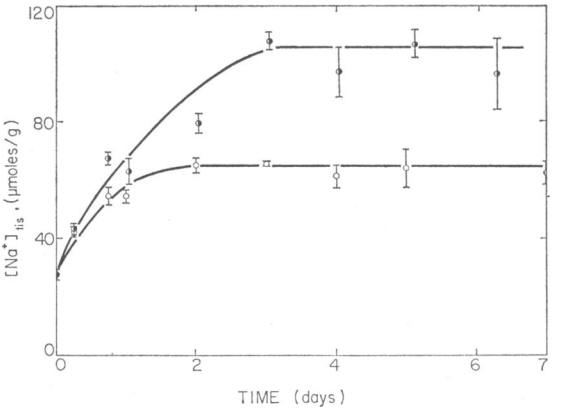

FIGURE 5 Time course of Na^+-ion content changes in frog muscles in media containing below normal K^+-ion concentrations. Media contained 100 mM Na^+-ion in both sets of data, but different K^+-ion concentration (0.1 mM [◑] and 0.7 mM [○]).

K^+-ion concentration is below normal, 72 hr being required at very low external K^+-ion concentrations (Figs. 4 and 5).

The Survival of Muscle Cells In Vitro in a Low K^+-Ion Environment

After the muscles are incubated for three days in a solution containing very low K^+-ion concentration and 100 mM Na^+ ion, the ionic contents of the muscle cells approach those in the bathing medium. Nevertheless, that the muscles are fully alive is demonstrated by the prompt and complete reversal to a normal ionic distribution pattern once the K^+-ion–depleted muscle is brought into a solution containing a normal concentration of K^+ ion (Fig. 6, see also below). One recalls that Steinbach demonstrated a similar reversal as far back as 1940 (See Steinbach, 1940).

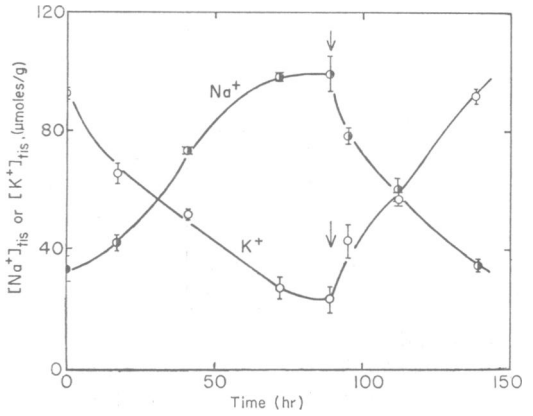

FIGURE 6 The demonstration of full reversibility of K^+-ion and Na^+-ion content changes in frog muscles. At 0 time muscles were introduced into a medium containing 0.2 mM K^+ ion and 100 mM Na^+ ion, and samples were taken out and analyzed. At the time indicated by the arrow, the remaining muscles in the flask were moved to a medium containing a normal K^+-ion concentration (2.5 mM) and 100 mM Na^+-ion. Complete reversal to a normal K^+-ion concentration was achieved in about 50 hr.

The Equilibrium Distribution of K^+ and Na^+ Ion in Muscle Cells at Varying External K^+-Ion Concentration and a Fixed Na^+-Ion Concentration

Linear Plots. Table I shows the results of a typical experiment and the various stages of calculations leading to the final results concerning the equilibrium K^+-ion and Na^+-ion concentrations in frog sartorius muscles[3] in a medium containing a fixed Na^+-ion concentration (100 mM) and K^+-ion concentrations varying from 0.05 mM to about 10 mM. A linear plot of the K^+ ion adsorbed against the external K^+-ion concentration reveals a sigmoid curve (Fig. 7). That is, at low external K^+-ion concentration, the adsorbed K^+-ion concentration is low. As the external K^+-ion concentration increases the adsorbed K^+-ion concentration rises sharply. The curve eventually levels off after the external K^+-ion concentration has reached approximately 2 mM. The solid line going through most of the experimental points is a theoretical curve according to equation 1 calculated on the following basis. The total number of adsorption sites, $[f]$, is equal to 120 μmoles/g of fresh cells; $K^{00}_{Na \to K}$ is equal to 100; and $-\gamma/2$ is equal 0.62 kcal/mole. The dotted line is a simple Langmuir adsorption isotherm with $[f] = 120$ mmoles/kg and a $K^{00}_{Na \to K}$ value of 100, which the experimental data do not fit as well.

The inset of Fig. 7 represents a plot of the total tissue K^+-ion concentration without any correction. This inset is included to show that the sigmoid shape of the curve is not artifically introduced by the correction factors for the extracellular space or interstitial ions.

[3] Without the aid of antibiotics and other techniques only recently available, earlier workers in this field were not able to keep alive isolated muscles long enough for new ionic equilibrium to be reached in the low K^+-ion concentration range. It is in the low K^+-ion concentration range, the cooperative nature of the uptake reveals itself. At high K^+-ion concentration, new equilibrium is reached more readily and the present data in these regions are in general agreement with those of Fenn and his coworkers (Fenn and Cobb, 1934; Fenn, Cobb, Hegnauer, and Marsh, 1934) and of Steinbach (1937).

TABLE I

EQUILIBRIUM K^+- AND NA^+-ION CONCENTRATIONS IN MUSCLE

The complete experimental data of the experiment performed on 12 August 1969 and illustrated in Figs. 7–9. Data are represented as mean ±SE.

Number of muscles	$[K]_{ex}$	$[K]_{tis}$	$[K^+]_{in}$	$[K^+]_{ad}$	$[Na^+]_{tis}$	$[Na^+]_{in}$	$[Na^+]_{ad}$	$\frac{[K^+]_{ex}}{[Na^+]_{ex}}$	$[K^+]_{ad}/[Na^+]_{ad}$
	(mM)	(μmoles/g)	(μmoles/g)	(μmoles/g)	(μmoles/g)	(μmoles/g)	(μmoles/g)		
4	0.05	1.95	2.09	2.06	121	122	114	0.0005	0.017
		±1.2	±0.65	±0.65	±0.75	±0.94	±6.1		±0.005
4	0.17	6.3	6.76	6.66	122	124	116	0.0017	0.054
		±2.1	±1.5	±1.5	±0.65	±0.40	±6.1		±0.12
4	0.40	10.2	10.9	10.7	115	116	108	0.0040	0.099
		±2.0	±0.68	±0.7	±3.0	±3.3	±6.8		±0.007
4	0.72	42.0	45.5	45.0	85.8	87.7	78.7	0.0072	0.577
		±3.9	±1.4	±1.4	±1.2	±3.4	±6.0		±0.04
4	1.18	49.0	52.8	51.9	70.0	67.0	59.1	0.0118	0.879
		±3.9	±1.7	±1.5	±1.8	±2.0	±4.8		±0.04
4	1.62	101	109	109	31.8	25.4	17.6	0.0162	6.37
		±5.8	±1.1	±1.1	±1.3	±1.5	±2.8		±0.67
3	2.34	102	112	111	31.6	26.6	17.3	0.0234	6.44
		±7.0	±4.7	±4.6	±1.8	±1.8	±3.5		±1.3
4	5.04	103	112	111	24.3	17.3	9.39	0.0504	15.6
		±6.0	±6.3	±6.2	±2.1	±2.3	±3.0		±4.2
4	9.73	105	113	113	18.7	10.7	3.81	0.0973	33.7
		±6.1	±6.3	±6.2	±0.67	±1.0	±1.7		±8.5

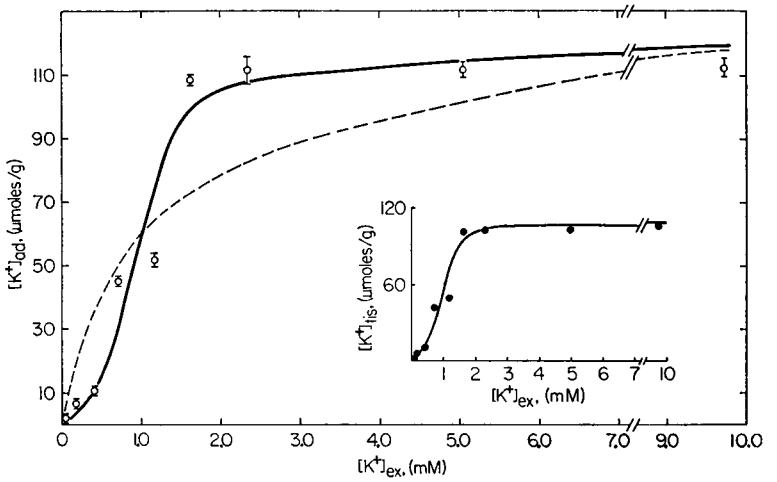

FIGURE 7 Linear plot of the adsorbed K^+-ion in frog muscle cells against equilibrium external K^+-ion concentration. The Na^+-ion concentration in the external medium was 100 mM in all cases. Equilibration time was 98 hr. Solid line is theoretically calculated according to equation 1 (see text). The dotted line is a Langmuir adsorption isotherm. In the inset are plotted the raw data of total tissue K^+-ion concentration without any correction. Date of experiment: 8-12-69. For complete numerical data, see Table I.

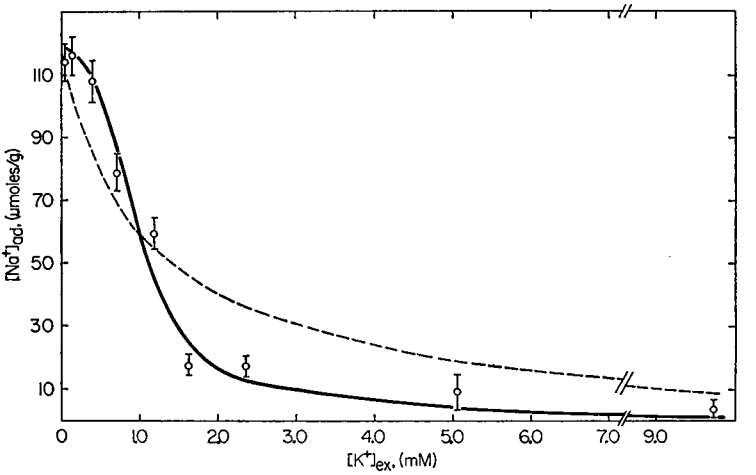

FIGURE 8 Linear plot of the adsorbed Na^+ ion in frog muscle cells against equilibrium external K^+-ion concentration. The external Na^+-ion concentration was constant at 100 mM in all cases. Equilibration time was 98 hr. The solid line is theoretically calculated according to equation 2 (see test). For complete numerical data, see Table I. Data from the same experiment shown in Fig. 7.

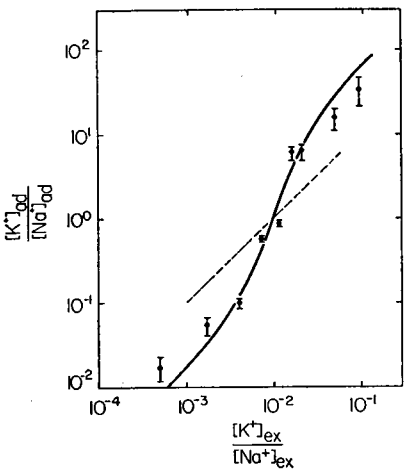

FIGURE 9 Log-log plot of the ratio of adsorbed K^+ and Na^+ ion against the ratio of equilibrium external K^+-ion and Na^+-ion concentration. The solid line is theoretically calculated according to equation 5 (see text). The dashed line is a Langmuir adsorption isotherm, a straight line with a slope of unity. The Na^+-ion concentration in the external medium was 100 mM in all cases. Equilibration time was 98 hr. Same experiment as described in Figs. 7 and 8.

Fig. 8 shows a linear plot of the adsorbed Na^+-ion concentrations in the same muscles against the equilibrium K^+-ion concentration in the bathing medium, the external Na^+-ion concentration being constant at 100 mM. Again, the best-fitting solid line is theoretically calculated according to equation 2, with the same values for $[f]$, $K^{00}_{Na \to K}$, and $-\gamma/2$ as those used to obtain the solid theoretical curve in Fig. 7. Again, the dotted line in Fig. 8, calculated according to a Langmuir adsorption isotherm with the same values of $[f]$ and $K^{00}_{Na \to K}$ as used in deriving the dotted line of Fig. 7, does not fit the data as well.

Log-Log Plot. The data presented in both Figs. 7 and 8 are combined in a log-log plot shown in Fig. 9. Here the ordinate represents the logarithm of the ratio of adsorbed K^+ ion and adsorbed Na^+ ion and the abscissa represents the logarithm of the ratio of equilibrium extracellular K^+-ion and Na^+-ion concentrations. The dotted straight line, which represents a Langmuir adsorption isotherm with a slope of unity, does not fit with the experimental data. Instead, the data fit much better with theoretical curves calculated according to equation 5, with the same values for $K^{00}_{Na \to K}$ and $-\gamma/2$ values used to derive the (solid) theoretical curves of Figs. 7 and 8.

Demonstration of the Reversible Nature of the Equilibrium Distribution

Fig. 6 shows that muscle deprived of most of its K^+-ion content by prolonged equilibrium in a medium containing a very low concentration of K^+ ion can revert to a normal K^+-ion content when placed once again in a Ringer solution containing the normal 2.5 mM of K^+ ion. In another series of experiments, we equilibrated muscle in low K^+-ion solution so that the K^+-ion concentration of muscles in the solution was brought to a value of 18.5 $\pm$2.0 μmoles/g of muscle tissues. These K-depleted muscles were then transferred into solution containing 100-mM Na^+-ion and varying K^+-ion concentrations and allowed to equilibrate for three more days.

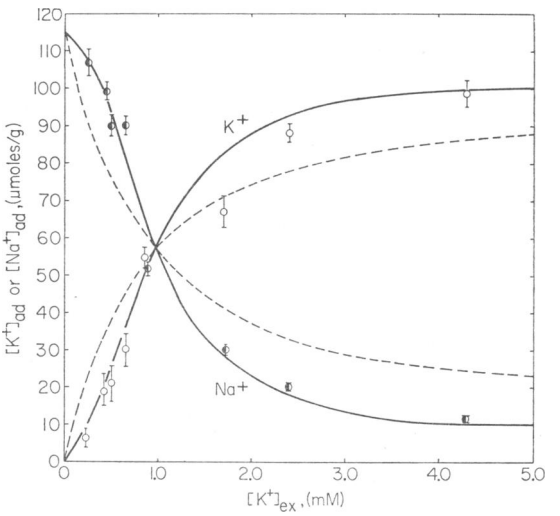

FIGURE 10 Linear plot of external K^+-ion and Na^+-ion concentrations against equilibrium adsorbed K^+-ion and Na^+-ion concentrations in frog muscle which had been previously depleted of its K^+-ion content. All muscles are equilibrated in K^+-free medium for 72 hr until their K^+-ion content was reduced to 18.5 $\pm$ 2.0 μmoles/g and their Na^+-ion content increased to 91.7 $\pm$ 4.5 μmoles/g. They were then equilibrated in media containing various K^+-ion concentrations but a constant Na^+-ion concentration (100 mM). The solid line is theoretically calculated according to equation 1, with $[f]$ = 110 mmoles/kg of fresh cells, $K^{00}_{Na \to K}$ = 109, and $-\gamma/2$ = 0.41 kcal/mole. The dotted line is a Langmiur adsorption isotherm with the same $[f]$ and $K^{00}_{Na \to K}$ values. Each point represents the mean $\pm$SE of four muscles.

TABLE II

SUMMARY OF DATA

Date	Duration of incubation	$[f]$	$K^{00}_{Na \to K}$	$\Delta F^{00}_{Na \to K}$	n	$-\gamma/2$
	(*hr*)	(*μmoles/g*)		(*kcal/mole*)		(*kcal/mole*)
7-9-64	89	117	133	2.89	3.6	0.76
7-17-64	66	110	152	2.98	2.78	0.61
7-20-64	70	95	170	3.04	2.48	0.54
7-21-64	72*	100	124	2.86	2.42	0.59
7-24-64	72	115	161	3.02	1.91	0.38
7-27-64	71.5	135	139	2.92	3.14	0.67
7-30-64	90	120	145	2.95	2.18	0.45
12-16-64	120	65	118	2.83	1.87	0.38
12-31-64	92	110	161	3.03	1.98	0.40
11-21-65	92	—	172	2.05	3.32	0.71
12-19-67	72*	115	109	2.78	2.05	0.41
3-26-68	72	108	124	2.87	2.25	0.47
8-12-69	98	120	100	2.70	2.89	0.62
Average ±SE		109 ± 4.8	135 ± 29	2.84 ± 0.07	2.53 ± 0.15	0.54 ± 0.04

* From reversal experiment.

The K^+-ion and Na^+-ion concentrations measured follow a pattern not appreciably different from those shown in Figs. 7 and 8. Thus, Fig. 10 shows that the K^+-ion distribution also follows a sigmoid curve. The solid line is theoretically calculated according to equation 1; $[f]$ is equal to 110 μmoles/g of fresh cells, $K^{00}_{Na \to K} = 109$, and $-\gamma/2 = 0.41$ kcal/mole. Similarly, the solid line for Na^+ ion was calculated according to equation 2 using a value of 120 μmoles/g for $[f]$, 109 for $K^{00}_{Na \to K}$, and $-\gamma/2 = 0.41$ kcal/mole.

Thus, there is accordance between Figs. 7, 8, and 10. This accordance shows that we are dealing with a reversible phenomenon.

In Table II, we have calculated all the sets of experimental data obtained during the last five years. The average value for $[f]$ is 109 μmoles/g, for $K^{00}_{Na \to K}$, 135, for $-\Delta F^{00}_{Na \to K}$, 2.84 kcal/mole, and for $-\gamma/2$, 0.54 kcal/mole. These values are close to those derived from the study of canine carotid arteries: $-\Delta F^{00}_{Na \to K} = 2.8$ kcal/mole and $-\gamma/2 = 0.61$ kcal/mole (Jones and Karreman, 1969 *a* and *b*).

DISCUSSION

Possible Interpretation of the Present Data in Terms of the Membrane-Pump Theory

The data obtained show a type of distribution curve for K^+ and Na^+ ion different from that predicted by that of a carrier-mediated distribution curve suggested for

various sugars (equations 12 and 13) or that dictated by the Donnan equilibrium (see Fig. 1 of Ling and Ochsenfeld, 1966). In both cases the theoretical distribution curves are hyperbolic or quasihyperbolic. However, this disagreement, as such, does not constitute a disproof of the Na-pump theory.

It is conceivable that further postulations can be made concerning the pumps or carriers such that a better fit with the data can be obtained. However, we have not attempted to make such postulations, because there are too many conflicting factors involved to construct a self-consistent theoretical model (see Ling, 1969).

In addition, we do not see how we can overcome the fundamental thermodynamic difficulties presented by the membrane-pump theory (Ling, 1962, 1965 *a*). Attempts were made recently to ascribe a major part of the Na^+-ion efflux rate to a non-energy-consuming exchange diffusion mechanism (Levi and Ussing, 1948; Essig, 1968; Keynes, 1966), thereby reducing the energy of the pump. However, experimental studies have shown such exchange diffusion does not exist in Sepia and Loligo nerves (Hodgkin and Keynes, 1955), nor in human red blood cells (Hoffman and Kregenow, 1966), nor in guinea pig smooth muscles (Buck and Goodford, 1966). Such evidence produced by Keynes and Swan (1959) that half of the total Na^+-ion efflux is due to exchange diffusion, is greatly weakened when Hoffman and Kregenow demonstrated that sensitivity to external Na^+-ion concentration, a criterion on which Keynes and Swan based their conclusion, is insufficient to establish the exchange diffusion mechanism.

Furthermore, we cannot reconcile the membrane theory with the recent NMR evidence that at low external K^+-ion and high external Na^+-ion concentration, the extra Na^+ ion accumulated is NMR-invisible and must therefore be in an adsorbed state (Ling and Cope, 1969). In fact, these findings offer further evidence in support of the association-induction hypothesis.

A Comparison of the Present Data with the Uptake of Oxygen by Erythrocytes

The oxygen uptake by erythrocytes represents such an old and familiar problem that one tends to forget the fundamental similarity between this phenomenon and the type of phenomenon described here. In both cases, we are dealing with the steady-level distribution of a solute in living cells. In both types of investigation, one continually varies the external concentration of one solute under study, while holding at a constant level the concentration of competing and noncompeting species, (e.g., water, Na^+ ion). In both cases we are dealing with a reversible phenomenon. In both cases we obtain a sigmoid-shaped distribution curve.

The story of oxygen distribution in erythrocytes shows that the cell membrane does not play a significant role in determining the steady level in the cell of this one solute. It is the major intracellular protein, hemoglobin, that controls the level of oxygen in the red blood cells. The oxygen uptake by erythrocytes is reproduced qualitatively at

least in a pure solution of hemoglobin (see Wyman, 1948; McFarlane and Robb-Smith, 1961).

Cooperative Shifts between a K^+-Ion and a Na^+-Ion State

Let us now examine the present data of K^+-ion and Na^+-ion uptake in terms of the association-induction hypothesis. Like oxygen uptake by hemoglobin, the uptake of K^+ and Na^+ ion is sigmoid. The mean intrinsic free energy of exchange, $-\Delta F^{00}_{Na\to K}$, is 2.84 kcal/mole. The average nearest-neighbor interaction energy, $-\gamma/2$, is 0.54 kcal/mole. This means that if all sites in a succession of sites are occupied by K^+ ion, then any one of those sites will prefer K^+ over Na^+ ion by (2.84 + 0.54) = 3.38 kcal/mole. We may refer to this protein as being in the *K^+-ion cooperative state.* If we lower the external K^+-ion concentration while keeping constant the Na^+-ion concentration, a point will be reached when the system tips over the steep part of the sigmoid-shaped curve and enters into a state where practically all the sites are occupied by Na^+ ion. In this *Na^+-ion state* the preference of each site for K^+ ion over Na^+ ion is no longer 3.38 kcal/mole but (2.84 − 0.54) = 2.30 kcal/mole. Thus, the data show that the sites adsorbing these alkali-metal ions can exist in two states in an all-or-none manner. The K/Na preference is greater in one state than in the other. The critical concentration of K^+ ion at which this transition occurs is about 2 mM (just below the normal extracellular K^+-ion concentration 2.5 mM). Thus, the normal muscle cell is in the K state, somewhat precariously poised, not too far from the critical threshold at which it shifts to the Na state.

It is pertinent to point out that Steinhardt and Zaiser (1951, 1953) have long since shown that carbonyl and ferrihemoglobin show a "zipper-like" release of many acidic groups when the external H^+-ion concentration reaches a certain critical concentration. In other words an autocooperative change of pK value from low to high occurs in response to a threshold concentration of H^+ ion (Ling, 1962, Chap. 7), much as the data we have presented may be described as an explosive release of K^+-ion binding sites when a critical K^+-ion concentration in the external medium is reached.

Why is the Na-K Distribution Curve Sigmoid in Shape while the K-Rb and K-Cs Distribution is Hyperbolic?

The association-induction hypothesis contends that it is the difference in the induction or electric polarization effect of the alternative adsorbents that sets off the sigmoid-shaped autocooperative phenomena. It was suggested that the nearest-neighbor interaction energy between a pair of adsorbents should be a function of the difference in the intrinsic free energy of adsorption between the two species (ΔF^{00}_{ij}) (Ling, 1962, Chap. 5).

The data shown in Table II give us a rough idea of the relation between ΔF^{00} and $-\gamma/2$. Thus, the average figure for $-\Delta F^{00}_{Na\to K}$ is 2.84 kcal/mole and that for $-\gamma/2$,

0.54 kcal/mole or about 19% of $\Delta F^{00}_{Na \to K}$. In a preceding paper on equilibrium ion distribution in frog sartorius muscles (Ling and Ochsenfeld, 1966), we have shown that when we studied Rb^+-ion distribution in the presence of K^+ ion, or Cs^+-ion distribution in the presence of K^+ ion, the (intrinsic) adsorption energies for K^+, Rb^+, and Cs^+ ion are − 3.85, − 3.92, and − 3.66 kcal/mole, respectively. Thus, the free energy difference $\Delta F^{00}_{Rb \to K}$ between K^+ and Rb^+ ion is (− 3.85 + 3.92) = +0.07 kcal/mole and $\Delta F^{00}_{Cs \to K}$ between K^+ and Cs^+ ion, (− 3.85 + 3.66) = −0.19 kcal/mole. If the same relation between $-\gamma/2$ and $\Delta F^{00}_{Na \to K}$ seen for K^+ and Na^+ ion applies, then $-\gamma/2$ would be (0.07 × 0.19) = 0.013 kcal/mole for the K^+-ion and Rb^+-ion interaction and (0.19 × 0.18) = 0.034 kcal/mole for the K^+-ion and Cs^+-ion interaction. Both are below the 2% experimental error, and beyond detection. Thus, theoretically, we would anticipate that the $-\gamma/2$ would not be observable and that the adsorption isotherm would be the Langmuir type. This is exactly what we have observed (Ling and Ochsenfeld, 1966).

APPENDIX

Method of Data Calculation

Elsewhere we have shown that a frog muscle like the sartorius can be divided into three basic components (Ling and Kromash, 1967; Ling, Neville, Will and Shannon, 1969).

(*a*) The muscle cell proper, including the cell interior from the plasma membrane inward, but not including the transverse tubules (T-system).

(*b*) The "connective tissue complex," which includes all types of connective tissues around and between the muscle fibers such as tendons, fascia, sarcolemma, small blood vessels, and small nerves. Together these constitute about 5% of the total fresh tissue weight (Ling, 1968). Fig. 11 shows the equilibrium uptake of K^+ and Na^+ ions by connective tissue sheets. These were equilibrated in modified Ringer solutions from whole sartorius muscle, as described in

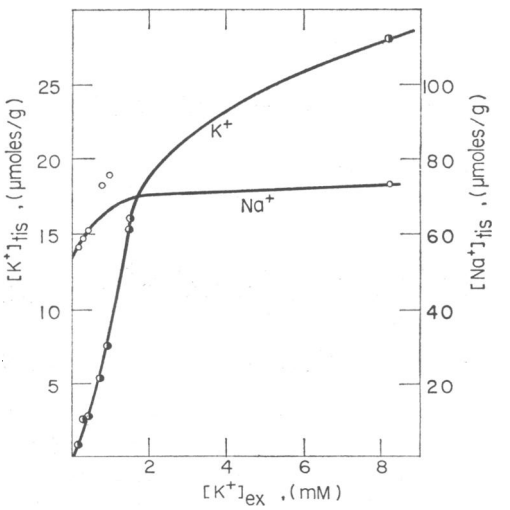

FIGURE 11 Equilibrium uptake of K^+ and Na^+ ion by connective tissue. Connective tissue from area adjacent to the sartorius muscle and incubated for the same length of time and in the same solution as the experimental muscle.

the Methods. The K^+-ion concentration $[K^+]_{ct}$ can be roughly described by two equations. For $[K^+]_{ex}$ under 2 mM,

$$[K^+]_{ct} = 10\,[K^+]_{ex}. \qquad \text{A1}$$

For $[K^+]_{ex}$ = 2–10 mM,

$$[K^+]_{ct} = 1.8\,[K^+]_{ex} + 13. \qquad \text{A2}$$

30% of fresh connective tissue is accessible to I-131–labeled serum albumin and to poly-L-glutamate (PLG). Hence, this portion belongs to the extracellular space proper assayed with PLG, as discussed next.

(*c*) The "extracellular spaces proper," which includes the spaces between the muscle fibers, the spaces between collagen and elastic fibrils, and the T-system. The concentration of ions in the extracellular space proper is the same as that in the external solution. The ceiling value for the extracellular space is 8% (Ling and Kromash, 1967); the best estimate is 5%. Since 30% of the connective tissue complex (which is [0.05 × 0.3] = 1.5% of the tissue weight) belongs to the extracellular space proper, it is already taken into account as part of the connective tissue complex. The extracellular space proper to be taken into account here is (5% − 1.5%) = 3.5%.

Formula for calculating the total intracellular K^+- and Na^+-ion concentrations

$$[K^+]_{in} = \frac{[K^+]_{tis} - 0.05\,[K^+]_{ct} - (0.05 - 0.05 \times 0.3)\,[K^+]_{ex}}{1 - 0.05 - (0.05 - 0.015)} \qquad \text{A3}$$

$$= 1.09\,\{[K^+]_{tis} - 0.05\,[K^+]_{ct} - 0.035\,[K^+]_{ex}\}. \qquad \text{A4}$$

For $[K^+]_{ex}$ under 2 mM, one substitutes equation A1 into equation A4 to obtain the following:

$$[K^+]_{in} = 1.09\,([K^+]_{tis} - 0.54\,[K^+]_{ex}). \qquad \text{A5}$$

For $[K^+]_{ex}$ from 2 to 10 mM

$$[K^+]_{in} = 1.09\,\{[K^+]_{tis} - 0.09\,[K^+]_{ex} - 13 \times 0.05 - 0.035\,[K^+]_{ex}\} \qquad \text{A6}$$

$$= 1.09\,\{[K^+]_{tis} - 0.13\,[K^+]_{ex} - 0.65\}. \qquad \text{A7}$$

On the other hand,

$$[Na^+]_{in} = \frac{[Na^+]_{tis} - 0.05\,[Na^+]_{ct} - (0.05 - 0.05 \times 0.3)\,[Na^+]_{ex}}{0.92} \qquad \text{A8}$$

$$+ 1.09\,\{[Na^+]_{tis} - 0.085\,[Na^+]_{ex}\}. \qquad \text{A9}$$

Formula for calculating total intracellular adsorbed ions. In preceding communications (Ling, 1965 *b*, 1969; Ling and Cope, 1969) we have presented evidence that intracellular Na^+ ion exists in two fractions: adsorbed, and dissolved in the cell water. The ion in the cell water, referred to as interstitial ion, has a concentration of about 10% of that in

the extracellular fluid, i.e., $\alpha q^{00\rightarrow ins} = 0.1$. The total water content of frog muscles is 80%. Of this about 8% belongs to the extracellular space proper and connective tissue complex. The total intracellular water in terms of weight per gram of muscle tissue is 0.72; in terms of weight per gram of muscle cells, it is (0.72/0.915) = 0.79 (ml/g); thus,

$$[Na^+]_{int} = \alpha q_{Na}^{\infty\rightarrow ins} [Na^+]_{ex} = 0.1 \times 0.79 [Na^+]_{ex} = 0.079 [Na^+]_{ex}. \quad A10$$

We have no direct measurement of $q_K^{\infty\rightarrow ins}$. However, a similar q value for Na^+, Rb^+, and Cs^+ ions was found in our model system, i.e., cellulose acetate membranes.[1] This suggests that $q_K^{\infty\rightarrow ins}$ and $q_{Na}^{\infty\rightarrow ins}$ may reasonably be assumed equal. Thus,

$$[K^+]_{int} = \alpha q_K^{\infty\rightarrow ins} [K^+]_{ex} = 0.079 [K^+]_{ex}. \quad A11$$

Proceeding from equations A9 and A10, and remebering that $[Na^+]_{ex}$ is constant at 100 mM we derive the formula for adsorbed Na^+ ion.

$$[Na^+]_{ad} = 1.09 \{[Na^+]_{tis} - 0.085 [Na^+]_{ex} - 0.079 \times 0.92 [Na^+]_{ex}\} \quad A12$$

$$= 1.09 \{[Na^+]_{tis} - 0.15 [Na^+]_{ex}\}. \quad A13$$

For $[K^+]_{ex}$ under 2 mM, we drive from equations A5 and A11

$$[K^+]_{ad} = 1.09 \{[K^+]_{tis} - 0.61 [K^+]_{ex}\}, \quad A14$$

and for $[K^+]_{ex}$ from 2 mM to 10 mM, from equations A7 and A12 we derive

$$[K^+]_{ad} = 1.09 \{[K^+]_{tis} - 0.20 [K^+]_{ex} - 0.65\}. \quad A15$$

John A. Hartford Foundation, Inc., N. Y., provided much of the basic equipment used in the investigation.

This investigation was supported by National Institute of Health Grant 2 RO1-GM11422-03, -04, -05, -06.
The senior investigator was also supported by Public Health Service Research Career Development Award K3-GM-19,032.

Received for publication 30 October 1969 and in revised form 10 February 1970.

REFERENCES

BOYLE, P. J., and E. J. CONWAY. 1941. *J. Physiol.* (*London*). **100**:1.
BUCK, B., and P. J. GOODFORD. 1966. *J. Physiol.* (*London*). **183**:551.
CHAPMAN, G., and K. A. MCLAUCHLAN. 1967. *Nature* (*London*). **215**:391.
COHEN, G. N., and J. MONOD. 1957. *Bacteriol. Rev.* **21**:169.
COPE, F. W. 1969. *Biophys. J.* **9**:303.
DANIELLI, J. F. 1954. *In* Recent Development in Cell Physiology. J. A. Ketching, editor. Butterworth & Co., (Publishers), Ltd., London. 1.
DEAN, R. B. 1941. *Biol. Symp.* **3**:331.
ERNST, E. 1963. Biophysics of the Striated Muscle. Hungarian Academy of Science, Budapest.
ESSIG, A. 1968. *Biophys. J.* **8**:53.
FENN, W. O., and D. M. COBB. 1934. *J. Gen. Physiol.* **17**:629.
FENN, W. O., D. M. COBB, A. H. HEGNAUER, and B. S. MARSH. 1934. *Amer. J. Physiol.* **110**:74.

FISCHER, M. H., and G. MOORE. 1908. *Amer. J. Physiol.* **20**:330.
FRITZ, O. G., and T. J. SWIFT. 1967. *Biophys. J.* **7**:675.
HAZLEWOOD, C. F., B. L. NICHOLS, and N. F. CHAMBERLAIN. 1969. *Nature (London).* **22**:747.
HILL, A. V. 1910. *J. Physiol. (London).* **40**:iv.
HODGIN, A. L., and R. D. KEYNES. 1955. *J. Physiol. (London).* **128**:28.
HOFFMAN, J. F., and F. M. KREGENOW. 1966. *Ann. N. Y. Acad. Sci.* **137**:566.
JONES, A. W., and G. KARREMAN. 1969 *a. Biophys. J.* **9**:884.
JONES, A. W., and G. KARREMAN. 1969 *b. Biophys. J.* **9**:910.
KARREMAN, G. 1965. *Bull. Math. Biophys.* **27**:91.
KEYNES, R. D. 1966. The Function of the Cell Membrane in "Myocardial Cell". S. A. Briller and H. L. Conn, editors. University of Pennsylvania Press, Philadelphia. 63.
KEYNES, R. D., and R. C. SWAN. 1959. *J. Physiol. (London).* **147**:591.
KOSHLAND, D. E., JR., G. NEMETHY, and D. FILMER. 1967. *Biochemistry.* **5**:365.
LEVI, H., and H. USSING. 1948. *Acta. Physiol. Scand.* **16**:232.
LING, G. N. 1952. Phosphorus Metabolism. W. D. McElroy and B. Glass, editors. The Johns Hopkins University Press, Baltimore. **2**:748.
LING, G. N. 1962. A Physical Theory of the Living State: The Association-Induction Hypothesis. Blaisdell Publishing Co., Waltham, Mass.
LING, G. N. 1964 *a. Tex. Rep. Biol. Med.* **22**:244.
LING, G. N. 1964 *b. Biopolym. Symp.* **1**:91.
LING, G. N. 1965 *a. Fed. Proc.* **24**:S103.
LING, G. N. 1965 *b. Ann. N. Y. Acad. Sci.* **125**:401.
LING, G. N. 1965 *c. Perspect. Biol. Med.* **9**:58.
LING, G. N. 1966. *Fed. Proc.* **25**:958.
LING, G. N. 1967. *In* Thermobiology. A. Rose, editor. Academic Press, Inc. New York. 5.
LING, G. N. 1968. *Food Technol.* **22**:1254.
LING, G. N. 1969. *Int. Rev. Cytol.* **26**:1.
LING, G. N., and G. BOHR. 1969. *Physiol Chem. Phys.* **1**:591.
LING, G. N., and F. W. COPE. 1969. *Science (Washington).* **163**:1335.
LING, G. N., and M. KROMASH. 1967. *J. Gen. Physiol.* **50**:677.
LING, G. N., M. C. NEVILLE, S. WILL, and P. SHANNON. 1969. *Physiol. Chem. Phys.* **1**:85.
LING, G. N., and M. M. OCHSENFELD. 1966. *J. Gen. Physiol.* **49**:819.
MCFARLANE, R. G., and A. H. T. ROBB-SMITH. 1961. Functions of the Blood. Academic Press, Inc. New York.
MONOD, J., J. WYMAN, and J. CHANGEUX. 1965. *J. Mol. Biol.* **12**:88.
OVERTON, E. 1907. *In* Handbuch der Physiologie des Menchen. W. Nagel, editor. Braunschweiger Mosik-Verlag Richard Maul, Braunschweig. **2**:744.
PFEFFER, W. 1921. Osmotische Untersuchungen. Engelmann, Leipzig. 2nd edition.
STEINBACH, H. B. 1937. *J. Cell Comp. Physiol.* **9**:429.
STEINBACH, H. B. 1940. *J. Biol. Chem.* **133**:695.
STEINHARDT, J., and E. M. ZAISER. 1951. *J. Biol. Chem.* **190**:97.
STEINHARDT, J., and E. M. ZAISER. 1953. *J. Amer. Chem. Soc.* **75**:1599.
TROSCHIN, A. S. 1958. Das Problem der Zellpermeabilität. Veb Gustav Fischer Verlag, Jena.
WILBRANDT, W., and T. ROSENBERG. 1961. *Pharmacol. Rev.* **13**:0109.
WYMAN, J. 1948. *Advan. Protein Chem.* **4**:407