STUDIES ON ION DISTRIBUTION IN LIVING CELLS: III. COOPERATIVE CONTROL OF ELECTROLYTE ACCUMULATION BY OUABAIN IN THE FROG MUSCLE

GILBERT N. LING and GRACE F. BOHR

Department of Molecular Biology, Division of Neurology, Pennsylvania Hospital, Eighth and Spruce Streets, Philadelphia, Pennsylvania 19107

(Received June 2, 1971)

SUMMARY

Ouabain (from 8×10^{-9} to 3.26×10^{-7} M) caused a decrease in the steady level of K^+-ion concentration in a dose-dependent manner. This effect was reversed by removing ouabain or by raising the external K^+-ion concentration. K^+ ion lost by the cells was seen to be replaced by Na^+. Na^+ ion accumulated in response to the drug treatment was shown by nuclear magnetic resonance spectroscopy to be predominantly in the adsorbed state. These results are interpreted quantitatively according to the association-induction hypothesis.

INTRODUCTION

In 1962, a general theory of cell function was published called the association-induction hypothesis.[1] With the use of NMR and other techniques, many of the predictions of this model have been confirmed. For instance, various evidence from several different laboratories shows that in adult muscle cells the bulk of cell water is abnormal and probably polarized.[2-7] The NMR technique has also provided evidence supporting the prediction that a major portion of Na^+ ion in the cell is complexed.[8-13] Studies using different techniques have confirmed this conclusion.[14-17] Recent advances in the NMR technique are now allowing investigation of the physical state of K^+ ion. Preliminary studies have suggested that this ion is also adsorbed,[18] in agreement with earlier conclusions from NMR studies of Na^+ ion in Kdepleted muscles.[14]

On the basis of theoretical analysis, the association-induction hypothesis suggested that the uptake of K^+ and Na^+ ions should follow a cooperative mechanism.[1,19] The accumulation of K^+ and Na^+ ion in frog muscle,[20,21] in vascular smooth muscle,[22,23] and in smooth muscle from the rabbit uterine myometrium[24] was seen recently to follow these predictions in a quantitative manner. It is the objective of this paper to extend the

approach used in these experiments and to describe experiments that illustrate a mechanism underlying the biologic control processes.

How this control can be induced via the action of cardinal adsorbents (e.g., drugs, hormones) through a cooperative mechariism was a part of the general theory described earlier.' Herein, a more specific model will be presented for the action of ouabain. The physiological significance of the approach of cooperative control of cell functions will be discussed, and the possibility of applying the association-induction hypothesis to quantitatively represent the effects of pharmacological agents will be established.

THEORY

A general equation for the distribution of solutes (e.g., K+-ion distribution in the presence of Na^+) in living cells may be written as:[2]

$$[p_i]_i = [p_i]_{int} + \sum_{L=1}^{N} [p_i]_{ad}^{L}, \tag{1}$$

where $[p_i]_i$, $[p_i]_{int}$, and $[p_i]_{ad}^{L}$ are the concentrations of total intracellular ith solute, interstitial ith solute in cell water, and adsorbed ith solute on the Lth type of sites among a total of N types of sites. For the simpler case where there is only one type of adsorption sites, Equation 1 can be explicitly written as:

$$[p_i]_i = a\, q_i\, [p_i]_{ex} + \frac{[f]}{2} \cdot \left(1 + \frac{\xi - 1}{\sqrt{(\xi-1)^2 + 4\,\xi \exp(\gamma/RT)}}\right), \tag{2}$$

where a is the percentage of water in the cell; q_i is the equilibrium distribution coefficient of the ith solute between the intracellular water hnd the external medium: [f] is the concentration of the adsorption sites; and γ is the free energy of nearest-neighbor interaction between the ith and jth solutes. ξ is defined as follows:

$$\xi = \frac{[p_i]_{ex}}{[p_j]_{ex}} \cdot k_{j\to i}^{oo}, \tag{3}$$

where $[p_i]_{ex}$ and $[p_j]_{ex}$ are, respectively, the equilibrium concentration of the ith and jth solutes in the external medium and $K_{j\to i}^{oo}$ is the intrinsic equilibrium constant for the ith and jth solute adsorption; it is related to the intrinsic standard free energy $\Delta F_{j\to i}^{oo}$ by

the equation:

$$\Delta F^{oo}_{j\to i} = RT \ln K^{oo}_{j\to i} . \quad (4)$$

When $-\frac{\gamma}{2}$ is positive, the tendency is for all the sites to be occupied by either the ith solute or the jth solute. This type of cooperative interaction is called *autocooperative*. These cooperative states are referred to as the "ith state" and the "jth state," respectively. In the present paper, the ith and jth solutes refer to K^+ and Na^+ ion, respectively; the ith state is then the K state and the jth state the Na state.

Another concept introduced in the association-induction hypothesis is that a "gang" of cooperatively linked sites (see Fig. 1) may shift from one state to another by the adsorption (and return to the original state by desorption) of a *cardinal adsorbent*.

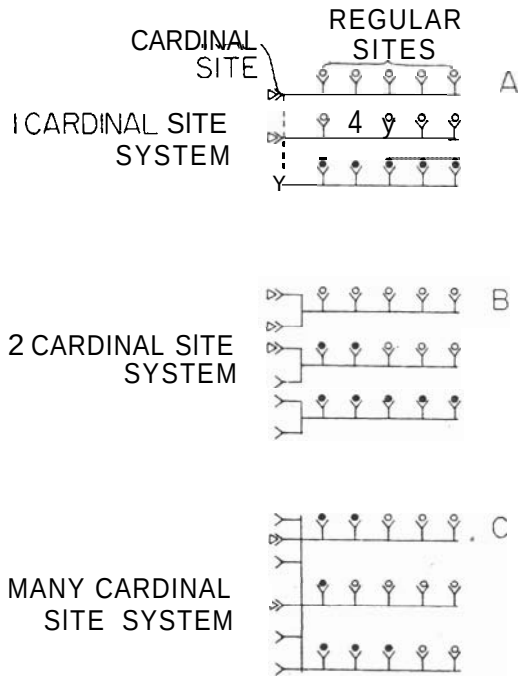

Figure 1. Diagrammatic Illustration of Cooperative Adsorption Controlled by Cardinal Sites. In A, there is one cardinal site for each gang of regular sites: in B, there are two cardinal sites; in C, there are many. Empty and filled circles represent alternative adsorbents on regular sites; triangle represents cardinal adsorbent.

This shift induced by the adsorption of a cardinal adsorbent, e.g., ouabain, can result in a change in the amounts of adsorbed K^+ and Na^+ ions and is represented by a change in value of the parameter $K^{oo}_{Na\rightarrow K}$. The cardinal adsorbent can initiate this change by inducing an alteration in the electron density at the sites adsorbing K^+ ion. This conformational alteration, expressed as a change in the c value of sites (ref. 1, p. 57), can alter the relative preference of the sites from K^+ towards Na^+ ion. This leads to an exchange of adsorbed K^+ for Na^+ ion and the process continues until the gang has exchanged all its K^+ for Na^+ ion.

The quantitative effects of different concentrations of cardinal adsorbents in changing the K^+ and Na^+ ion in gangs of sites may be described as follows:

(1) One-Cardinal-Site System: Let us consider a system of protein chains, each containing one gang of g cooperatively linked regular sites under the control of a single cardinal site, as illustrated in Figure 1A. Each gang in this case consists of five regular sites, i.e., g = 5. If the total concentration of gangs in the system is N, N is then equal to the concentration of cardinal sites, [F]. The general equation for the concentration of adsorbed cardinal adsorbent $[C]_{ad}$ is:

$$[C]_{ad} = \frac{[F]}{2}\left(1 + \frac{\Xi - 1}{\sqrt{(\Xi - 1)^2 + 4\,\Xi \exp(\Gamma/RT)}}\right), \tag{5}$$

where Ξ is defined in a manner analogous to ξ in Equation 3.

$$\Xi = [C]_{ex} \cdot \mathcal{K}^{oo}_{c}. \tag{6}$$

Here the alternative adsorbent on the cardinal site is unspecified but, being a constant, it is adsorbed in $\mathcal{K}^{oo}_{c}$, the adsorption constant of the cardinal adsorbent. $-\frac{\Gamma}{2}$ is the nearest-neighbor interaction among the cardinal sites. (The dashed line in Fig. 1A indicates the direction of this interaction.)

Now, according to the association-induction hypothesis, the adsorption of the cardinal adsorbent changes the value of $K^{oo}_{j\rightarrow i}$; it may also change $-\frac{\gamma}{2}$. Thus, we shall now define two sets of symbols: ξ_c, $K^{oo}_{j\rightarrow i(c)}$ and $-\frac{\gamma_c}{2}$ referring to gangs where the cardinal site is occupied; ξ_o, $K^{oo}_{j\rightarrow i(o)}$ and $-\frac{\gamma_o}{2}$ referring to gangs where the cardinal site is vacant.

The general equation for the ith solute adsorption in the one-cardinal-site system is:

$$[p_i]_{ad} = \frac{g[F]_c}{2}\left\{1 + \frac{\xi_c - 1}{\sqrt{(\xi_c - 1)^2 + 4\,\xi_c \exp(\gamma_c/RT)}}\right\} + \frac{g[F]_o}{2}\left\{1 + \frac{\xi_o - 1}{\sqrt{(\xi_o - 1)^2 + 4\,\xi_o \exp(\gamma_o/RT)}}\right\} \quad (7)$$

where $[F]_c$ and $[F]_o$ are the concentration of occupied and vacant cardinal sites, respectively, $[F]_c = [C]_{ad}$, and $[F]_o = [C]_{ad}$.

Figure 2 shows theoretical plots of $X_i = \frac{[p_i]_{ad}}{g([F]_c + [F]_o)}$, as obtained from Equation 7, with $-\frac{\Gamma_c}{2}$ and $-\frac{\Gamma_o}{2} = 0$. Note that before the cardinal adsorbent reaches a very high concentration, each curve has two separate plateau regions. Theoretical curves for the case where both $-\frac{\Gamma_c}{2}$ and $-\frac{\Gamma_o}{2}$ are greater than zero were recently presented elsewhere.[25]

Theoretical values of $[p_j]_{ad}$ can be readily obtained from Figure 2 as $[p_j]_{ad} = g[F] - [p_i]_{ad}$.

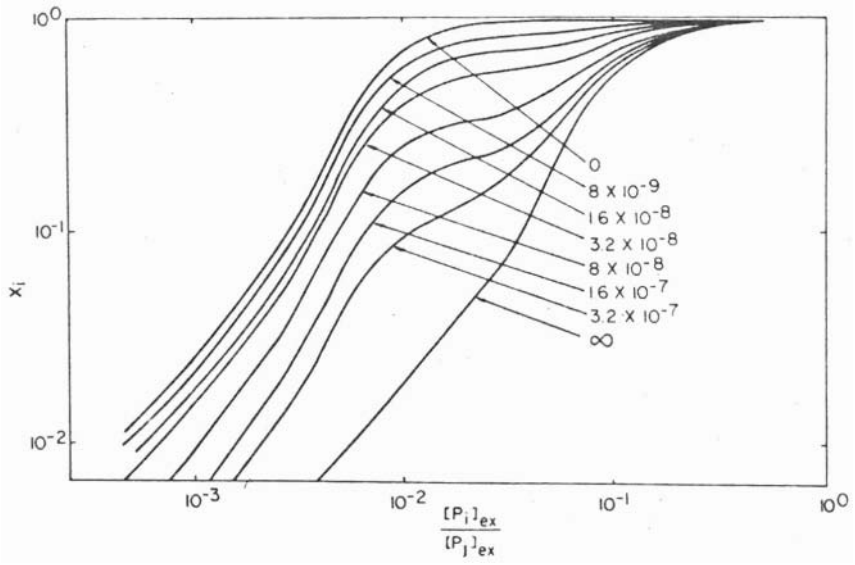

Figure 2. Control of the ith Solute Adsorption by Different Concentrations of a Cardinal Adsorbent at Different ith (and jth) Solute Concentrations in a One-Cardinal-Site System.
Theoretical curves calculated on the basis of $K_c = 4 \times 10^{-8}$ M, $K^{oo}_{(j\to i)o} = 7 \times 10^{-3}$ M and $K^{oo}_{(j\to i)c} = 9 \times 10^{-2}$ M. $-\frac{\gamma_o}{2} = \frac{\gamma_o}{2} = 0.6$ Kcal/mole. Numbers in chart refer to molar concentrations of cardinal adsorbent in the external medium. Ordinate represents mole fraction of adsorption sites occupied by the ith solute. Abscissa represents the ratio of ith and jth solute in the medium.

(2) Two-Cardinal-Site System: In the case where there are two cardinal sites controlling each gang (as shown in Fig. 1B), N is equal to $\frac{[F]}{2}$. There will be three different sets of symbols: (a) ξ_{oo}, $K^{oo}_{j\to i(oo)}$, and $-\frac{\gamma_{oo}}{2}$ values corresponding to gangs with both cardinal sites vacant; (b) ξ_{cc}, $K^{oo}_{j\to i(cc)}$ and $-\frac{\gamma_{cc}}{2}$ values corresponding to gangs with both cardinal sites occupied; and (c) ξ_{co}, $K^{oo}_{j\to i(co)}$, and $-\frac{\gamma_{co}}{2}$ corresponding to gangs with only one cardinal site occupied.

The equation for the total concentration of the ith adsorbed solute is:

$$[p_i]_{ad} = \frac{g[F]_{cc}}{2}\left\{1+\frac{\xi_{cc}-1}{\sqrt{(\xi_{cc}-1)^2+4\,\xi_{cc}\exp(\gamma_{cc}/RT)}}\right\} + \frac{g[F]_{co}}{2}\left\{1+\frac{\xi_{co}-1}{\sqrt{(\xi_{co}-1)^2+4\,\xi_{co}\exp(\gamma_{co}/RT)}}\right\} + \frac{g[F]_{oo}}{2}\left\{1+\frac{\xi_{oo}-1}{\sqrt{(\xi_{oo}-1)^2+4\,\xi_{oo}\exp(\gamma_{oo}/RT)}}\right\}, \quad (8)$$

where $[F]_{cc}$, $[F]_{co}$, and $[F]_{oo}$ refer to the pair of cardinal sites belonging to each gang, being both occupied, one occupied, and both vacant, respectively.

The mole fraction of cardinal sites in the three different states is (see in ref. 26):

$$X_{cc} = \frac{[F]_{cc}}{F} = \frac{1}{1+\frac{K_{cc}}{LC]_{\text{„}}}+\frac{K_{cc}K_{co}}{[C]^2_{ex}}} \quad (9)$$

$$X_{co} = \frac{[F]_{co}}{F} = \frac{1}{1+\frac{[C]_{ex}}{K_{cc}}+\frac{K_{co}}{[C]_{ex}}} \quad (10)$$

$$X_{oo} = \frac{[F]_{oo}}{F} = \frac{1}{1+\frac{[C]_{ex}}{K_{co}}+\frac{[C]^2_{ex}}{K_{cc}K_{co}}}, \quad (11)$$

where K_{cc} and K_{co} are the dissociation constant of the first and second stage of dissociation of the cardinal site adsorption:

$$F_{cc} \longrightarrow F_{oc} \longrightarrow F_{oo}.$$

Figure 3 shows a theoretical plot for $X_i = \frac{[p_i]_{ad}}{g([F]_{cc} + [F]_{co} + [F]_{oo})}$ and $X_j = 1 - X_i$ as obtained from Equation 9. Comparison of Figure **3A** with Figure 2 reveals that with an increase in the number of cardinal sites for each gang, the plateaus as seen in Figure 2 become less distinct.

(3) Many-Cardinal-Sites System: When there are more than two cardinal sites per gang, the equation governing the control of adsorption on regular sites becomes more complex. However, the trend is toward a smooth transition of the $K^{oo}_{j\to i}$ values between the extreme cases of complete occupancy of all the cardinal sites and complete vacancy; the weighed average of $K^{oo}_{j\to i}$ varies with the concentration of adsorbed cardinal adsorbent.

The simplest case to consider is that in which the cardinal site adsorption follows the Langmuir isotherm (no near-neighbor interaction) and in which the departure of $\Delta F^{oo}_{j\to i}$, $\Delta\Delta F^{oo}_{j\to i}$ is linearly related to the concentration of adsorbed cardinal adsorbent: Thus,

$$\Delta\Delta F^{oo}_{j\to i} = \frac{\sigma[F]\, K_c\, [C]_{ex}}{1 + K_c\, [C]_{ex}}, \tag{12}$$

where σ is a proportionality constant. With the aid of Equations 3, 4, and 12, we now can calculate the concentration of adsorbed ith solute from Equation 2. The theoretical curves calculated are shown in Figure 4.

MATERIALS AND METHODS

All experiments were performed at 25°C on sartorius muscles isolated from Wisconsin and Vermont leopard frogs (*Rana pipiens pipiens*, Schreber). Ouabain was obtained from the Sigrna Chemical Company, St. Louis.

The methods of sterile tissue dissection and of long-term preservation have been recently described elsewhere.[27] For extraction and analysis of K^+ and Na^+ ion, the hot HCl method was used.[27] The procedure used for the preparation of a Ringer-GIB solution containing a fixed concentration of Na^+ ion (100 M) but a varying concentration of K^+ ion (0.02 to 10 M) has been described in the same article.

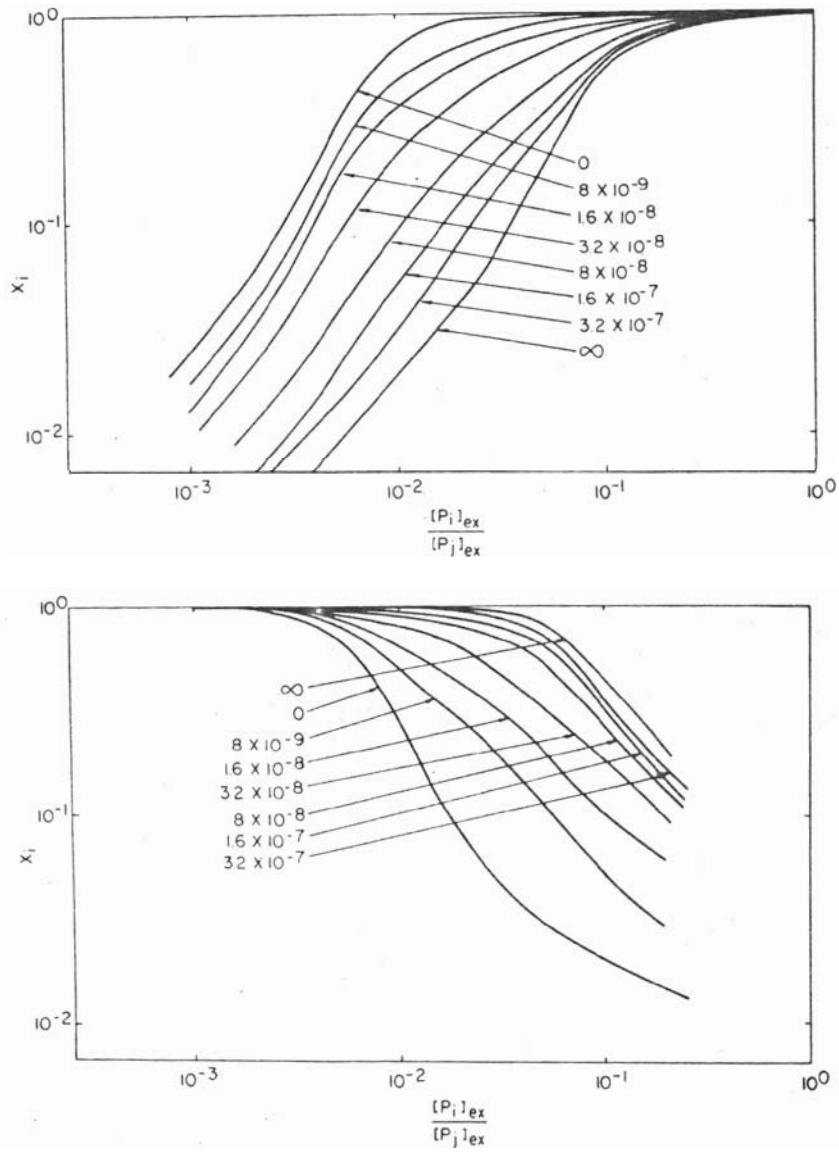

Figure 3. Control of (A) ith and (B) jth Solute Adsorption by Concentrations of the Cardinal Adsorbent at Different ith (and jth) Solute Concentrations in a Two-Cardinal-Site System.
K_{cc} = 1.8 x 10^{-8} M; K_{co} = 4 x 10^{-8} M. $K^{oo}_{(j\to i)oo}$, $K^{oo}_{(j\to i)oc}$, and $K^{oo}_{(j\to i)cc}$ are respectively 7 x 10^{-3}, 2.5 x 10^{-2} and 9 x 10^{-2} M. $\frac{\gamma_{oo}}{2} = \frac{\gamma_{oc}}{2} = \frac{\gamma_{cc}}{2}$ = 0.6 Kcal/mole. Numbers in chart refer to concentrations in moles/liter of cardinal adsorbent in the external medium. Ordinate and abscissa referred to same as described under Figure 2.

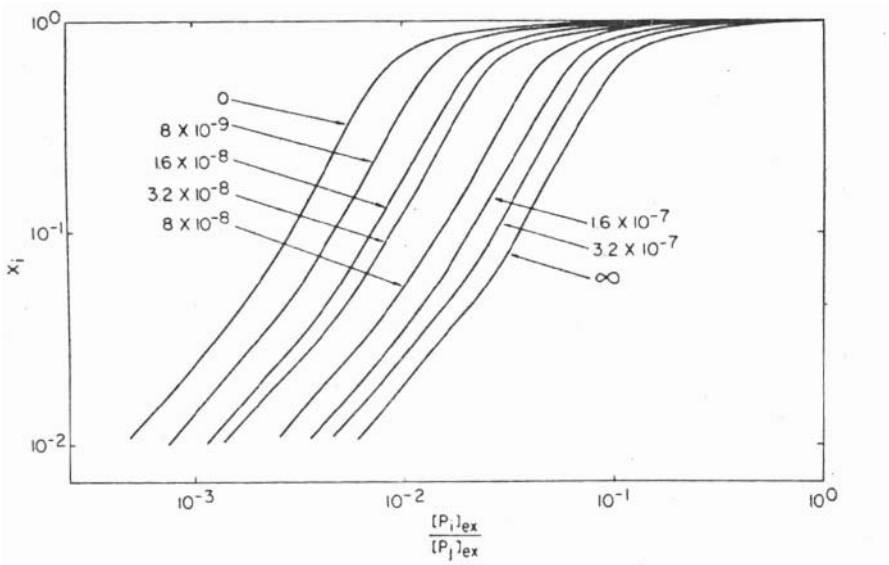

Figure 4. Control of ith Solute Adsorption by Cardinal Adsorbent at Different ith (and jth) Solute Concentrations in a Manycardinal-Site System.
$K_c = 4 \times 10^{-8}$ M. $K^{oo}_{(j \to i)}$ (all cardinal sites empty) = 7×10^{-3} M. $K^{oo}_{(j \to i)c}$ (all cardinal sites occupied) = 9×10^{-2} M. $-\frac{\gamma_o}{2} = -\frac{\gamma_c}{2} = 0.6$ Kcal/mole.

Nuclear magnetic resonance study of free and adsorbed Na^+ ion was performed in cooperation with Dr. Freeman W. Cope at Johnsville, Pa., using a wide-line Varian NMR spectrometer. The method was that described by Cope.[8]

ATP was extracted with a slight modification[23] of the method described by Cain and Davies.[28] Analysis was made enzymatically using the Sigma Chemical Company's Kit No. 366-W. ATP content is measured with the kit as a decrease of W adsorption at 340 mμ which occurs due to the oxidation of DPNH to DPN.

Ouabain at the highest concentration reported in this paper (3.26×10^{-7} M) lowered the K^+-ion concentration in connective tissues to about one half its normal value. This change, however, was so small that it fell within the experimental error. The effect of ouabain on Na^+-ion concentrations in the connective tissues was even smaller. Consequently, we used the same formulas for calculating the concentrations of intracellular and adsorbed K^+ and Na^+ ions as those presented in the preceding report of this series.[21]

RESULTS

Control of Electrolyte Accumulation by Ouabain

A. The Time Course of Ouabain Action and Its Reversibility: It is shown in Figure 5A that when ouabain (**3.3** x 10^{-7} M) is added to the bathing medium containing normal K^+, muscles lose K^+ and gain Na^+ ion. At 25°C, equilibrium values for both ions were reached in two to four days, depending on shaking rates and other conditions, and were maintained for at least two more days. It is next shown (Fig. 5B) that the effect of ouabain on the K^+- and Na^+-ion distribution was reversible. After three days of incubation in the presence of ouabain, the muscles were returned to the normal medium. The cells were seen to recover by losing Na^+ ion and gaining K^+ ion.

B. The Effects of Ouabain on the Equilibrium Concentration of K^+ and Na^+ Ions: With a fixed concentration of ouabain in the presence of varying external K^+-ion concentrations, $[K^+]_{ex}$, it is found that the effect of the drug is diminished or eliminated by increasing $[K^+]_{ex}$. This is shown in Table 1.

The above results for the adsorbed fraction of K^+ and Na^+ ion are plotted in Figure 6 and can be compared with the control values (without ouabain). The curve is seen to shift to the right in the presence of the drug. A higher value of $[K^+]_{ex}$ is required with ouabain to reach half saturation for the adsorption of K^+ ion on the sites. In other words, the $K^{oo}_{Na\to K}$ value is decreased with ouabain (from a control value of 100 to 17 with **3.3** x 10^{-7} M ouabain).

The results for the above and intermediate doses of ouabain are shown in Figures 7 and 8. There is seen to be a continuous shift in the $K^{oo}_{Na\to K}$ value that decreases with increasing doses of ouabain. A comparison of these Figures with the theoretical curves in Figures 2 and 3 reveal that the results are better described by a dual or multi-cardinal-site model. A single-cardinal-site model would show a plateau near the half-saturation point at the intermediate ouabain concentration levels. This is not observed.

NMR Study of Na in the Oriabain-Treated Muscles

The present action of ouabain is seen to decrease the $K^{oo}_{Na\to K}$ value. That is, at a fixed $[K^+]_{ex}$, the preference for K^+ over Na^+ ion is diminished and in consequence, part of the adsorbed K^+ ion is exchanged for Na^+ ion. Thus, if it can be shown that the exchanged Na^+ ion is indeed complexed in the cell, this would provide additional evidence in favor of the above theoretical mechanism for the action of ouabain.

An NMR signal for Na^+ ion in 10^{-7} M ouabain-treated muscle was obtained; the fraction of "invisible" and "visible" sodium contents are shown in Table 2. It is seen that the fraction of "invisible" Na^+ ion is increased in the treated muscles. Similar results were also obtained in the muscles that had been incubated in K^+-free solutions and were Na^+-ion rich. The invisible fraction of Na^+ ion represents the amount of Na^+ ion that is com-

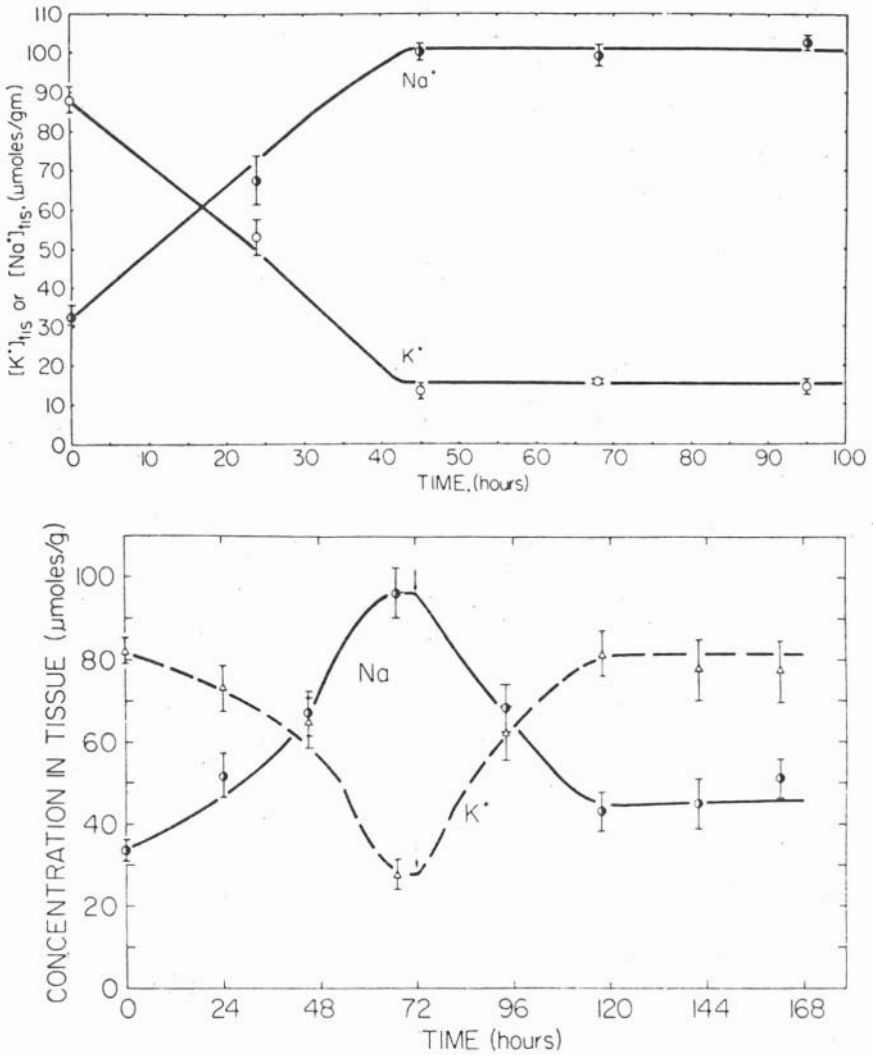

Figure 5A. Time Course of K^+ and Na^+ Ion Concentration Change in Response to the Addition of Ouabnin.
Ouabain at a final concentration of 3.26×10^{-7} M was introduced into the Ringer-CIB medium ($[Na^+]_{ex}$ = 100 mM; $[K^+]_{ex}$, 2.5 mM) bathing the sartorius muscles. Four muscles were taken out each time at different time intervals and analyzed for both their K^+ and Na^+ ion contents. The results are shown as mean ± standard error of the mean.

Figure 5B. Reversibility of the Ouabain Effect on the Na^+-Ion Distribution.
Ouabain at a final concentration of 10^{-7} M was introduced at 0 time, into a normal Ringer-GIB medium. After 72 hours: the muscles were rinsed and then transferred to ouabain-free medium which was changed every day in following days.

Table 1. Detailed Data of K^+ and Na^+ Ion in Muscles Treated with 3.26 x 10^{-7} M. Ouabain. $[K^+]_{ex}$, $[K^+]_{tis}$, and $[K^+]_{ad}$ refer to external tissues, intracellular and adsorbed K^+-ion concentrations, respectively, Similar symbols were used for Na^+ ion. Last column represents the sum of the concentrations of adsorbed K^+ and Na^+ ion.

$[K^+]_{ex}$ (mM)	$[K^+]_{tis}$ (μmoles/g)	$[K^+]_{ad}$ (μmoles/g)	$[Na^+]_{tis}$ (μmoles/g)	$[Na^+]_{ad}$ (μmoles/g)	$[K^+]_{ad}$ and $[Na^+]_{ad}$ (μmoles/g)
25.0	108.9 ± 6.5	113.2 ± 1.1	21.7 ± 2.0	12.6 ± 2.8	125.3 ± 2.8
20.0	100.2 ± 1.5	104.1 ± 1.7	26.45 ± 1.8	14.3 ± 1.9	123.7 ± 1.6
15.12	86.9 ± 5.4	91.6 ± 5.9	27.6 ± 2.2	13.1 ± 2.3	104.6 ± 7.0
8.00	72.5 ± 3.6	77.4 ± 4.2	44.1 ± 3.8	31.0 ± 4.1	110.9 ± 3.4
5.50	54.5 ± 2.4	58.3 ± 2.6	70.7 ± 3.1	60.2 ± 3.3	118.5 ± 1.8
3.31	11.5 ± 0.4	11.9 ± 0.6	106.3 ± 1.0	98.7 ± 1.0	110.5 ± 1.4
1.94	7.9 ± 0.4	7.3 ± 0.4	116.9 ± 4.1	106.1 ± 2.8	115.9 ± 2.8
1.02	4.0 ± 0.5	3.61 ± 0.5	121.2 ± 1.7	112.4 ± 4.0	115.9 ± 4.2
0.75	2.7 ± 0.4	2.34 ± 0.4	122.2 ± 1.0	115.8 ± 1.3	118.2 ± 1.6
0.59	1.06 ± 0.54	1.35 ± 0.6	117.2 ± 2.2	108.0 ± 3.6	109.5 ± 3.8
0.05	2.3 ± .071	2.10 ± 0.7	117.7 ± 1.8	111.2 ± 2.0	113.3 ± 2.6

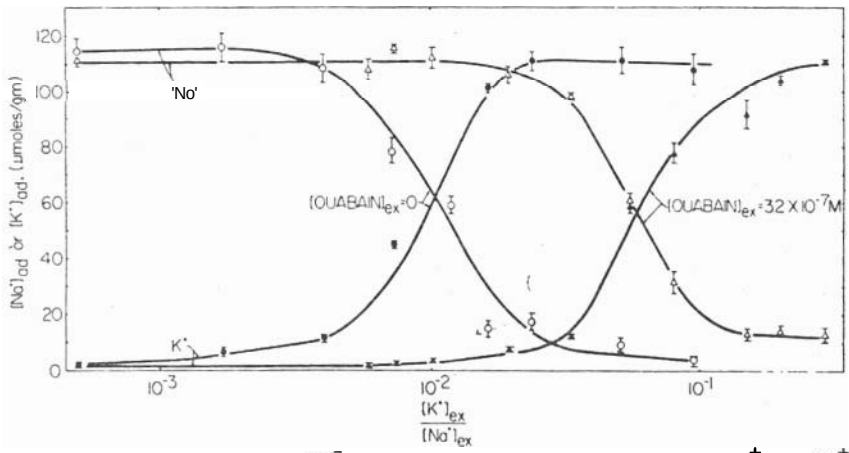

Figure 6. Effect of Ouabain (3.2 x 10^{-7} M) on the Equilibrium Distribution of K^+ and Na^+ Ion. Curves with open (Na^+) and filled (K^+) circles were equilibrium distribution data from muscles not treated with ouabain. The point of intersection gives a $K^{oo}_{Na\rightarrow K}$ of 100. In muscles treated with ouabain (3.2 x 10^{-7} M), $K^{oo}_{Na\rightarrow K}$ has shifted to 21.7.

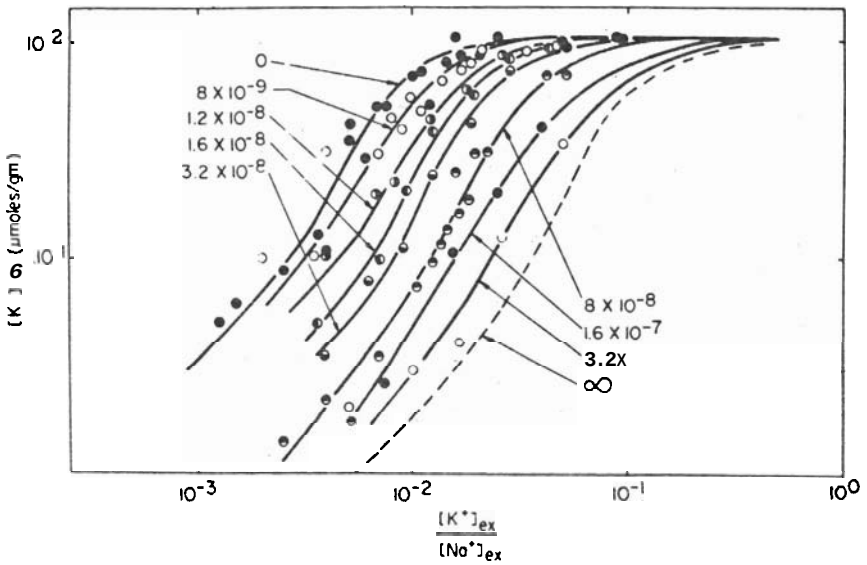

Figure 7. The Effect of Different Concentrations of Ouabain on the Equilibrium Distributions of K^+ Ion in Frog Muscle.
Each point is the average of 4 determinations. Standard errors, not shown to avoid confusion, are about the same as shown in Figure 6. Numbers in this and the following figure refer to equilibrium molar concentrations of ouabain in the external media.

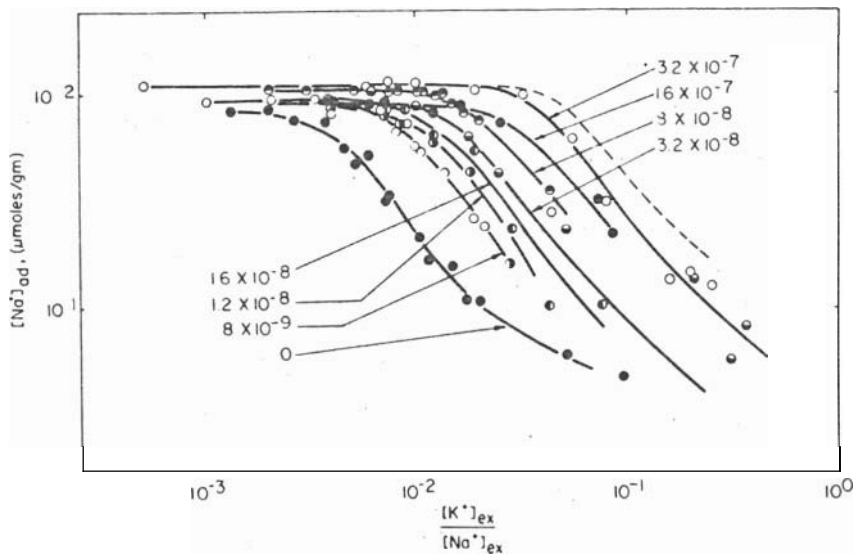

Figure 8. The Effect of Different Concentrations of Ouabain on the Equilibrium Distribution of Na^+ Ion in Frog Muscles.
Each point is the average of 4 determinations. Standard errors, not shown to avoid confusion, are about the same as shown in Figure 6. Data derived from the same muscles as those yielding the data of Figure 7.

plexed. These results are therefore interpreted to mean that Na^+ ion is replacing the adsorbed K^+ ion.

There is also a certain increment in the visible (free) Na^+ ion as well. The precise cause for this increment of NMR visible Na^+ ion is unexplained at present. It is attractive to speculate that the increment in the amount of visible Na^+ ion may be related to a possible decrease in the resting potential. Another possibility is that ouabain may cause a loosening of the water structure which would increase the interstitial Na^+ ion via a change in the q value (see Eq. 2).

Table 2. NMR Analysis of Free and Adsorbed Na^+ Ion in Normal, K-Depleted and Ouabain-Treated Frog Muscles.
Four types of small frog voluntary muscles were used (sartorius, semitendinosus, tibialis anticus longus and iliofibularis). Approximately 2 grams of muscles were used for each sample. Total K^+ and Na^+ ion contents were analyzed using flame photometry. NMR "invisible" Na was obtained by difference between total Na and NMR "visible" Na. Data of control and K-depleted muscles were taken from ref. 14 for comparison.

	Total K^+ (μmoles/kg)	Total Na^+ (μmoles/kg)	NMR Visible Na^+ (μmoles/kg)	NMR "Invisible" Na^+ (μmoles/kg)	Sum of NMR-"Invisible" Na^+ and Total K^+ (μmoles/kg)
CONTROL	78.4	25.0	10	14	93
	44.5	77	25	52	97
DEPLETED	37.8	56.9	24	33	71
	40.8	98.2	28	70	111
	38.0	78	25	53	91
OUABAIN	43.6	81.6	26	56	99.6
	49.1	81.2	24	57	106

The ATP Content of Ouabain-Treated Muscle

Figure 9 shows that at concentrations between 8×10^{-9} and 3.26×10^{-7} M, ounbain produced a profound change of K^+- and Na^+-ion contents in muscle cells; indeed, at 3.26×10^{-7} M the ouabain action was nearly complete. A further increase in ouabain concentration produced no further change in the K^+-ion and Na^+-ion concentrations. Within the range of ouabain concentrations (8×10^{-9} to 3.26×10^{-7} M) there was no significant change in the ATP contents in these muscle tissues. A dip from 4.2 to 2.5 μmole/g of

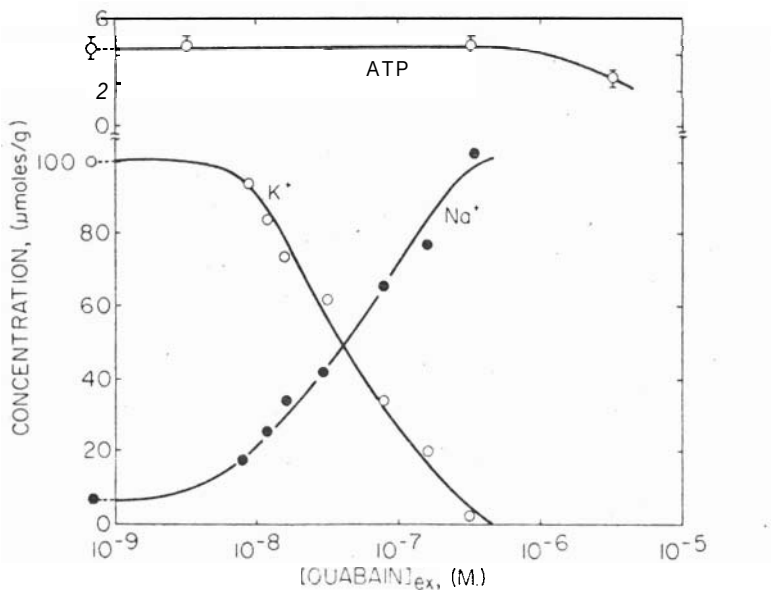

Figure 9. Effect of Different Concentrations of Ouabain on the K^+, Na^+ and **ATP** Contents of Frog Sartorius Muscles.
Muscles were esposed to different concentrations of ouabain in a Ringer-CIB medium (100 mM Na^+; 2.5 mM K^+) for three days and analyzed for its **ATP** contents. K^+ and Na^+ ion contents were taken from the data shown in Figures 7 and 8.

fresh tissue in **ATP** concentration occurred at an external ouabain concentration of 3.25 x 10^{-6} M. That ouabain at concentrations up to 3.26 x 10^{-7} M produces no significant effect on the **ATP** content simplifies the interpretation of the ouabain action. Therefore, it is suggested that the mechanism of the ouabain action on electrolyte accumulation is independent of metabolism. A similar conclusion has been made for the action of this drug on ion fluxes[29-31] and on contraction.[32]

DISCUSSION

The above results show that the basic changes produced by ouabain up to 3.26 x 10^{-7} M constitute a predictable shift in the preference of the cell for K^+ and Na^+ ion. The NMR data confirm that this shift in preference is a shift for selective adsorption of Na^+. In terms of the association-induction hypothesis, this implies that there is a decrease in the value of intrinsic equilibrium constant, $K^{oo}_{Na \to K}$.

It was possible to represent the effects of ouabain by its effect on a single parameter ξ

in Equation 2. Its value shifted, for instance, from ξ_{oo} to ξ_{co} or ξ_{cc} if one or both of the cardinal sites or a gang was filled. We know that

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

At a fixed $[K^+]_{ex}$ and $[Na^+]_{ex}$, the shift in ξ is determined by a change in the $K^{oo}_{Na \to K}$ value. As described earlier, the shift in $K^{oo}_{Na \to K}$ value is a measure of the change in the electronic conformation of the sites. Thus, we regard our findings as suggesting that the control of ouabain action on the electrolyte metabolism involves conformational changes in proteins.'

The reversibility of the ouabain action by K^+ ion can also be understood through the above relation (Eq. 3). In order to keep ξ unchanged, $[K^+]_{ex}$ (with fixed $[Na^+]_{ex}$) must be increased to compensate for the decreased value of $K^{oo}_{Na \to K}$. Our findings show that this mechanism is applicable for the action of ouabain.

The model presented is therefore seen to describe the cardinal control of electrolyte metabolism via cardiac glycosides in a quantitative manner. This approach was also used to describe the control of reversible oxygen binding on hemoglobin by another set of cardinal adsorbents (2,3-DPG, inosine hexaphosphate and ATP).[25] This physiologically important control process was shown to occur through the binding of the cardinal adsorbents on a single site per gang (Fig. 1A).

It was shown that ATP can act as a cardinal adsorbent in the control of electrolyte metabolism in frog muscle.[1,2,23] It was also shown that cooperative control of electrolyte levels in smooth muscle can be exerted by calcium and ouabain[23] and progesterone.[24] Collectively then, the evidence is considered to establish, quite convincingly, the importance of this biophysical approach in understanding the cellular behavior and its control via physiological and pharmacological agents. These findings, as discussed above, are also considered to exemplify the fundamental molecular mechanisms underlying the coordinated activities of protoplasm that set apart the living from the non-living.

ACKNOWLEDGMENT

We thank Dr. Freeman Cope, Dr. Jagdish Gulati and Miss Geraldine Smith for invaluable help. The John A. Hartford Foundation, Inc., New York, provided much of the basic equipment used in this study, which was otherwise supported by NIH Research Grant 2-ROl-GM11422-06, -07 and a contract with the ONR [Nonr-4371(00)]. G. N. Ling was supported by Public Health Service Research Career Development Award K3-GM-19.032.

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