Cat Heart Muscle *In Vitro*

IV. *Inhibition of transport in quiescent muscles*

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ABSTRACT Cellular concentrations, $[K]_i$, $[Na]_i$, and $[Cl]_i$, and cell water contents were measured *in vitro* at 27°C in cat papillary muscles. Measurements were made with and without ouabain at varying concentrations of K and ouabain, at pH 5.2 and 9.0, in absence of O_2, and in NaCl-free solution. Large losses of cell K and increases of cell Na occurred in presence of ouabain, at 2–3°C, and in K-free medium. The dependence of inhibition of cation transport by ouabain on external K concentration, studied at constant initial $[K]_i$, was consistent with a competition between K and ouabain localized to the external face of the membrane. In NaCl-free sucrose solution $[K]_i$ remained at its physiological value and was not affected by exposure to ouabain or low temperature, except when Ca was also omitted. Ouabain inhibition persisted at pH 9.0 and in Ca-poor media. Cells swelled and lost K at pH 5.2, and residual ouabain effect was small. At pH 9.0, or in absence of O_2, or in Ca-poor solutions cells became permeable to mannitol. The ion movements observed after inhibition of active transport are compatible either with a passive K distribution and a primary inhibition of Na extrusion or with inhibition of a coupled active transport of both K and Na.

Previous papers in this series (1, 2) have been concerned with the behavior of the cat right ventricular papillary muscle in the steady state when the electrochemical gradients across the cell membrane are held constant for the duration of the experiment. The present study deals with net movements of ions and water when this steady state is disturbed by inhibiting ion transport against the electrochemical gradient. The effect of ouabain on cell volume and cellular concentrations of K, Na, and Cl has been examined as a function of the external concentrations of the K, Na, H, and Ca ions and compared with the changes resulting from oxygen deprivation, from lowered temperature, and from exposure to K-free solution.

METHODS

Experimental Procedure

The experiments were designed to determine the changes in cell volume and cellular ion concentrations after a period of time long enough for net movements of ions and water to approach completion. This experimental procedure was adopted to minimize the effect of the differences in diameter of papillary muscles on the net fluxes. A 3 hour period of exposure to the experimental solution was chosen because the control muscles have already been shown to remain in a steady state with respect to cellular ion concentrations (1) and transmembrane resting potential difference (2) during this period. Under these conditions the differences between the control and treated muscles were large and uniform in all cases.

Papillary muscles from cats anesthetized with pentobarbital were dissected in the chamber of Page and Solomon (1) and incubated in the apparatus for perfusion with small volumes of solution described by Page (3). The two papillary muscles obtained from each heart were preincubated for 1 hour at 27–28°C in the reference solution to permit recovery from the preparative procedure. The muscles were then transferred to a medium labeled with inulin-C^{14}–carboxylic acid, mannitol-methyl-H^3, and Cl^{36}, ouabain being added to one incubation vessel and the other serving as a control. After 3 hours in these solutions, muscles were removed from the incubation vessels and analyzed for water content, inulin, mannitol, Cl^{36}, and K and Na contents as previously described (3). For the experiments in Ca-free solution and at altered pH, the muscles were transferred from the preincubation medium (the reference solution) to the altered solution for 30 minutes before adding radioactive tracers and ouabain. In this way the extracellular space was allowed to come into diffusion equilibrium with the bathing medium prior to exposure to the inhibitor. The effect of changes of temperature (in absence of ouabain) was studied in a series of muscles transferred after preequilibration at 27–28°C to baths at 17–18°C and at 2–3°C. For the experiments at 2–3°C, the usual 3 hour incubation period was extended to 4 hours, to compensate for possible slowing of permeation and diffusion at this temperature. Muscles were not stimulated and spontaneous activity was not observed except transiently in Ca-free solutions.

Some of these experiments were repeated in solutions with 5.32 mM K in which all the NaCl was replaced by isosmolar sucrose. Exposure to these solutions was limited to 2 hours because of their low ionic strength. A final series of experiments was designed to examine net ion movements in absence of external K. For this purpose two muscles from the same heart were first preequilibrated in physiological cat Ringer's solution (1), then transferred to an isosmolar medium without K. After 90 minutes, one muscle was removed for analysis, the other being returned for an additional 90 minutes to the K-containing preincubation medium to determine the reversibility of the observed effects.

Solutions

The basic solution, to be referred to as the reference solution, was a bicarbonate Ringer's solution with 2.5 mM K, isosmolar with solutions previously used (1, 2). It was selected because its K concentration was the lowest at which the transmembrane resting potential difference remained constant for a period of hours, indicating that a steady state with respect to intracellular ion concentrations could be maintained. Its composition (in mM) was Na 181.3, K 2.5, Cl 163.1, HCO_3 22, HPO_4 0.59, H_2PO_4 1.45, Ca 1.40, Mg 0.56, and glucose 5.5. For some experiments the K concentration was raised to 5.32 or to 25 mM by a mole for mole substitution of KCl for NaCl or lowered to zero by similarly substituting NaCl for KCl. In one series of experiments Ca was omitted from the solution. To test the effects of lowering the pH to 5.2, an acetate-phosphate buffer was prepared by addition of HCl to Na acetate, the final composition of the medium being (in mM) Na 175.5, K 2.5, Cl 170.4, acetate 10, acetic acid 2, H_2PO_4 1.5, Ca 1.4, Mg 0.56, glucose 5.5. The corresponding experiments at pH 9.0–9.1 were carried out in a medium buffered with tris(hydroxymethyl)-aminomethane (tris), the composition (in mM) being Na 178.5, K 2.5, Cl 182.9, HPO_4 1.0, tris 5.0, Ca 1.40, Mg 0.56, and glucose 5.5. This solution was bubbled with 100 per cent O_2 instead of with the usual 95 per cent O_2–5 per cent CO_2 mixture. The Ca and Mg concentrations are lower than the stated values because of the precipitation of Ca and Mg salts, which were removed by filtration.

The effect of removal of external Na was tested in a sucrose medium isosmolar with the reference solution, bubbled with 100 per cent O_2, and having a K concentration of 5.32 mM. Its composition (in mM) was K 5.32, H_2PO_4 0.205, HPO_4 1.295, Ca 1.40, Mg 0.56, Cl 6.3, sucrose 357, and glucose 5.5. Muscles subsequently immersed in this medium were preincubated in the physiological cat Ringer's solution previously described (1), made by substituting K for Na in the reference solution to raise the external K concentration to 5.32 mM.

Ouabain, U. S. P. (S. B. Penick and Co., New York), was stored in the cold and dissolved immediately before use.

RESULTS

Net Ion and Water Movements Due to Ouabain

Because cellular concentrations depend on the size of the molecule used to measure the extracellular space (3), they have been determined using both inulin and mannitol. Table I, which presents the cell volumes and extracellular spaces obtained under various conditions, contains the information necessary to derive the cell ion contents given in subsequent tables on which the discussion will be based. Mannitol spaces are included only if their behavior was of special interest. In Tables I, II, and IV, the results for the control muscles in the reference or modified solutions are compared with those in the same solutions in presence of ouabain. In addition, the effect of a particular modification of the medium may be obtained from these tables by

TABLE I

CELL WATER CONTENTS AND EXTRACELLULAR SPACES

Experimental modification	Cell water*	Extracellular space	
		Inulin	Mannitol
	kg/kg dry weight	*per cent of total water*	
Reference			
Control	1.91±0.07	35±1	48±1
Ouabain 10^{-5} M	2.56±0.08	30±2	49±2
25 mM K			
Control	2.32±0.08	26±1	—
Ouabain 10^{-5} M	3.22±0.12	24±3	
Reference			
Control	1.93±0.07	32±3	—
Ouabain 10^{-6} M	2.41±0.41	28±2	
25 mM K			
Control	2.10±0.12	29±3	—
Ouabain 10^{-6} M	2.27±0.15	28±1	
Ca-poor			
Control	1.58±0.06	45±2	82±4
Ouabain 10^{-6} M	2.09±0.17	48±4	81±3
pH 9			
Control	1.79±0.07	39±2	63±2
Ouabain 10^{-6} M	2.09±0.07	32±1	48±4
pH 5.2			
Control	2.45±0.04	32±1	—
Ouabain 10^{-6} M	2.57±0.12	30±2	
Nitrogen			
Control	1.93±0.07	42±2	65±2
Ouabain 10^{-6} M	2.47±0.15	33±2	53±2
17–18°C	2.22±0.06	30±2	—
2–3°C	2.52±0.07	28±2	—

* Cell water calculated on basis of inulin spaces.

comparing the controls in the modified medium with the controls in the reference solution (top line, Tables I, II, and IV). Table I shows that for the reference solution the values for extracellular spaces are comparable in control and ouabain-treated muscles, indicating that inhibition of ion transport by this cardiac glycoside is not associated with an increased permeability to extracellular carbohydrate molecules.

In evaluating net ion movements when cell concentrations and volume change simultaneously, it is convenient to work with the cellular content of ions referred to the dry weight. Tables II to VI in the succeeding sections of the paper give the ion contents expressed in this way, calculated by multiplying the cellular concentrations derived as previously described (1) by the cell water content per kilo dry weight. Results in all tables have been tabulated as mean ± standard error. Unless otherwise indicated, differences are described as significant if $P < 0.01$, and as not significant if $P > 0.05$, using the Student *t*-test.

The net ion movements in 10^{-5} M and 10^{-6} M ouabain, shown in Table II, are in the direction expected from inhibition of cellular extrusion of Na and uptake of K against the electrochemical gradients for these ions. These net ion movements do not appreciably alter the dry weight, and it is assumed that the experimental procedure does not cause significant changes in other substances contributing to the dry weight. Assuming that at physiological or higher pH Cl enters the cell accompanied by Na, the net Na influx may be partitioned into a fraction entering as NaCl and a fraction entering in exchange for K leaving the cell. The Na gained by the cell in exchange for K may then be compared with the total loss of cellular K in order to determine whether a K-Na exchange can account quantitatively for the net K efflux. For 10^{-5} M ouabain the excess of K lost over Na calculated as gained in exchange for K may be significant ($P < 0.05$, Student *t*-test); for 10^{-6} M ouabain the two quantities do not differ significantly, and the loss of cell K is reasonably well accounted for by an exchange with Na.

Table II shows that at a ouabain concentration of 10^{-6} M the net ion movements in 2.5 mM K Ringer's solution are still large, although somewhat smaller than in 10^{-5} M ouabain. When the muscle is preequilibrated in an isosmolar solution in which the external K concentration is raised tenfold to 25 mM by substitution of K for Na, $[K]_i$ in the control muscles remains unchanged, as previously shown (1). It therefore becomes possible to study the effects of ouabain as a function of $[K]_o$ at constant initial $[K]_i$. Under these conditions any first order interaction between ouabain and K should be localized to the face of the cell membrane exposed to the external solution, provided that the mechanism of action of ouabain is independent of cell volume, $[Cl]_i$, and the transmembrane potential difference (V_m). In Table II the net K and Na movements in 25 mM K solution are seen to be large after exposure to 10^{-5} M ouabain. However, when muscles preincubated in 25 mM K are treated with 10^{-6} M ouabain, the net ion movements produced by this glycoside are almost completely inhibited. The changes in cell Na and Cl contents are no longer statistically different from zero and the loss of cell K becomes very small. These results are compatible with an inhibition by K of a reaction between

ouabain and a surface site localized to the external face of the membrane or to a phase within the membrane in which the K concentration is in equilibrium with the extracellular solution.

Effects of Removal of External K

If ouabain interferes with an interaction between external K and the cell membrane, it should be possible to simulate the effects of ouabain by removal of external K. This hypothesis was tested by comparing 10 pairs of muscles exposed for 90 minutes at 27° to a K-free medium, one muscle being subsequently allowed to recover for 90 minutes in a physiological solution with

TABLE II
EFFECT OF OUABAIN ON CELL ION CONTENT AS FUNCTION OF $[K]_o$*

	$[K]_o$	K	Na	Cl
	mM		*mmols/kg dry weight*	
Control (11)	2.5	343±9	142±18	73±7
Ouabain 10^{-5} M		106±11	449±27	194±11
Control (8)	25	404±15	91±7	112±4
Ouabain 10^{-5} M		225±23	418±34	264±24
Control (8)	2.5	315±8	119±10	75±10
Ouabain 10^{-6} M		175±10	304±23	131±3
Control (8)	25	377±13	89±13	95±13
Ouabain 10^{-6} M		361±13	103±8	102±4

* In this and subsequent tables cell ion content is calculated using inulin space data of Table I, from which corresponding figures for mannitol space data can be calculated. The number of experiments is given in parentheses.

5.32 mM K. Table III indicates that the net movements of K and Na resulting from removal of external K do indeed simulate the effect of ouabain. The table shows that muscles have undergone a reversible loss of cell K, not statistically different from a similarly reversible gain in cell Na, without associated changes in cell volume or Cl content. These findings resemble those reported by Steinbach (4, 5) and others (6, 7) for frog skeletal muscle.

Effects of Ca-Poor Solutions

Since ouabain inhibition appears to depend on the interaction with K ions at a site on the external face of the cell membrane, the dependence of this phenomenon on pH was examined. pH values higher than physiological depress the activity of ionized Ca; it therefore seemed desirable to study sepa-

rately the effects of omission of Ca from the medium at the pH of the control solution (pH 7.3–7.4). The medium used will be referred to as a Ca-poor solution because it seems unlikely that the extracellular Ca concentration was actually reduced to zero. In this solution the mannitol space of the control muscles was found to be 82 per cent of total muscle water, indicating that the cell membrane had become permeable to this carbohydrate. The mannitol space cannot therefore be used in the measurement of cell volume. Moreover, the inulin space increased from a value of 35 per cent of total water in Ca-containing solution to 45 per cent, a figure approaching that of the mannitol space in the more physiological solution. It would therefore appear that omission of Ca renders portions of the extracellular compartment, which normally equilibrate very slowly, if at all, with inulin, accessible to penetration by the large inulin molecule. At the same time, cell water content, using the inulin space (kilo of cell water per kilo of dry weight), fell from a normal value of

TABLE III
EFFECT OF REMOVAL OF EXTERNAL K (27°C)

Bathing medium	Cell K	Cell Na	Cell Cl	Cell H_2O	Inulin space
		mmols/kg dry weight		*kg/kg dry weight*	*per cent of total water*
90 min. in K-free solution	283±15	183±18	84±3	2.24±0.03	31±2
Additional 90 min. recovery in 5.32 mM K solution	365±11	114±6	94±7	2.18±0.04	32±1

1.91 to 1.58 in the Ca-poor solution. Although, as shown in Table IV, the cell K content decreased significantly in the Ca-poor medium, the calculated value for $[K]_i$ of 169 ± 6 mmols/kg cell water is not significantly different from the corresponding value of 181 ± 5 in the reference solution. A diminution of cell volume and tissue K content without a fall in $[K]_i$ suggests that a fraction of K and water assumed to be cellular in the usual calculation of intracellular ion concentrations is lost in Ca-poor solutions. It is difficult to distinguish between a loss of cellular K affecting all cells, a shift in the partition of K between the cellular and extracellular compartments affecting all cells, and a selective loss of cellular K by a limited number of cells. A point against the last alternative is the report of Hoffman and Suckling (8), who found V_m of dog papillary muscle to be unaffected or to show a slight increase over a period of hours, even on addition of the chelating agent EDTA. A statistical analysis of the net changes in cell ion contents based on the inulin space data (Table IV) shows that gains in cell Na and Cl, unlike cell K loss,

are not significant. However, in view of the profound changes in the characteristics of the extracellular compartment, conventional calculations of cellular concentrations must be accepted with reserve. The changes in carbohydrate penetration raise the possibility of changes in passive membrane permeability which have been described in a number of excitable tissues in low Ca solutions (9). In addition, Ca-poor or Ca-free solutions are commonly used as an adjunct in methods of disaggregating heart muscle cells (10), suggesting that Ca ions may have important effects on the charge of the cell surface (11) and of extracellular connective tissue.

It is evident from Tables II and IV that net ion movements are more pronounced in 10^{-6} M ouabain solutions with Ca omitted than in a tenfold greater

TABLE IV

EFFECT OF OUABAIN INHIBITION IN ACID, ALKALINE, AND Ca-POOR MEDIA ON CELL ION CONTENT*

	Modification of medium	K	Na	Cl
		mmols/kg dry weight		
Reference	Unmodified‡	343±9	142±18	73±7
Control (12)	Ca-poor	267±12	196±25	96±12
Ouabain		55±12	527±54	259±12
Control (9)	pH 9	310±12	139±11	116±10
Ouabain		146±15	304±20	126±13
Control (8)	pH 5.2	286±10	182±29	154±8
Ouabain		236±12	221±19	183±22

* $[K]_o$ = 2.5 mM, concentration of ouabain 10^{-6} M.
‡ Line 1, Table II.

ouabain concentration in presence of Ca. Apparently the deficiency of Ca does not interfere with the inhibition of transport by ouabain. These enhanced effects of ouabain in Ca-poor solutions may in fact reflect an increased passive permeability of the cell membrane. Such a permeability change would facilitate diffusion of K and Na down their electrochemical gradients and tend to increase net efflux of K and influx of Na.

Effects of Altered pH

At pH 9.0 the value of the mannitol space lies between that in the control solution and that in the Ca-poor medium, suggesting that the ionized Ca concentration has likewise assumed an intermediate value. As in frog skeletal muscle (12), $[K]_i$ and $[Na]_i$ do not change at pH 9.0. It is apparent from

Table IV that ouabain inhibits transport at least as effectively at this pH as at pH 7.3.

The results in acid solution are very different. Incubation at pH 5.2 gives rise to a significant decrease in cell K content and significant increases in cell contents of Cl and water. Although a net entry of Na seems likely, the apparent elevation in cell Na content cannot be established as statistically significant because of the dispersion of the data.

If the K lost by the cell is considered as having exchanged for Na, the large cellular Cl uptake and the cell swelling remain to be accounted for. The concentration of H is too small for an appreciable quantity of Cl to enter as HCl, so that the cellular water uptake cannot be explained as water osmotically following this solute. Moreover, the concentration of H is also too small to account for cellular K loss in terms of K-H exchange, and an attribution of cell swelling to a diffusion of NaCl into the cell. An attractive hypothesis is an inhibition at low pH of the combination by K with the transport mechanism which appears to be localized to the external face of the cell membrane. In addition, it is necessary to consider the possibility that a fall in cellular pH may be associated with a change in the concentration of a cellular indiffusible anion, or with the production of osmotically active indiffusible hydrolysis products of ATP or creatine phosphate.

Addition of 10^{-6} M ouabain to muscles preincubated for 30 minutes at pH 5.2 produces a significant additional loss of cell K. Since cell water content does not change appreciably under these conditions, the K ion leaving the cell presumably does so in exchange for Na, although because of the dispersion of the data for Na, the apparent increase in cell Na content is again not significant.

Effects of Removal of Oxygen

In muscles incubated in an atmosphere of N_2 (95 per cent N_2, 5 per cent CO_2), the mannitol spaces are large, so that cellular concentrations and cell volumes are calculated for inulin space data only. The cell ion contents in millimoles per kilo dry weight after incubation with N_2 were, K 199 ± 27, Na 278 ± 46, Cl 133 ± 23. These values approximate those in muscles exposed for the same period to 10^{-6} M ouabain (Table II). The rather large dispersions for the muscles treated with nitrogen are illustrated in Fig. 1. Some muscles are characterized by high $[K]_i$, low $[Na]_i$, and low $[Cl]_i$, and others by low $[K]_i$, high $[Na]_i$, and high $[Cl]_i$. A distribution of this type might occur if the dependence of the active transport mechanism on oxygen were indirect. It seems probable that oxygen is required for the maintenance of cellular reserves of ATP, creatine phosphate, or some closely related energy-rich intermediate, and that impairment of active transport takes place only when the cellular reserves of these compounds become depleted. The observed dis-

tribution suggests that the depletion may have a fairly sharply defined threshold beyond which rapid and large fluxes of K and Na down their respective electrochemical gradients are precipitated. The proximity to this threshold for a given muscle would be a function of the reserves of energy-rich compounds at the beginning of the 3 hour incubation in N_2. Such reserves are well known to be extraordinarily labile in mammalian heart muscle (13, 14).

After simultaneous exposure to N_2 and 10^{-6} M ouabain the cell ion contents of papillary muscles (millimoles per kilo dry weight) were, K 114 ± 8, Na 375 ± 15, and Cl 177 ± 8. Under these conditions the K content is significantly lower and the content of Na significantly higher than in the con-

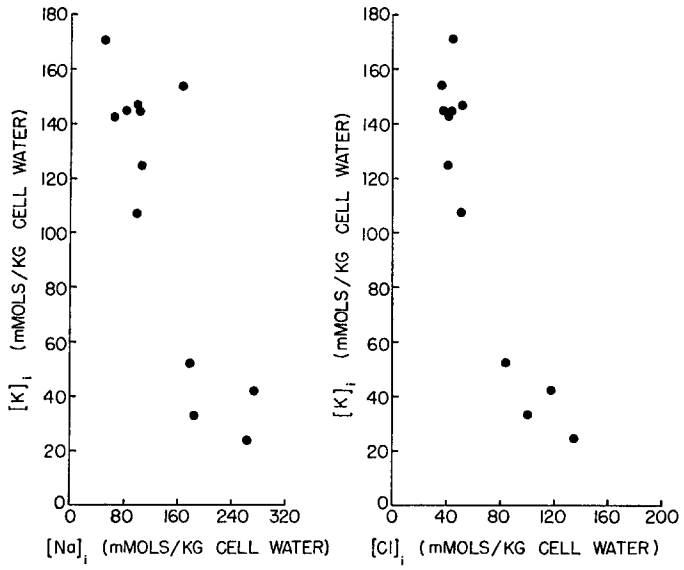

FIGURE 1. $[K]_i$ as a function of $[Na]_i$ and $[Cl]_i$ after 3 hours in nitrogen.

trols exposed to N_2 only and their values approximate those of muscles treated with 10^{-5} M ouabain in oxygenated Ringer's solution (Table II). A rather surprising observation was a large reduction in the mannitol space of muscles treated simultaneously with ouabain and N_2 as compared to N_2 alone. A similar effect of ouabain on the mannitol space is evident at pH 9.0 (Table I). These results suggest that ouabain may inhibit the increased permeability to mannitol under such conditions, although it apparently does not do so under the conditions prevailing in the Ca-poor medium.

Net Ion Movements at Lowered Temperature

A convenient method of slowing ion transport against the electrochemical gradient is to lower the temperature. Our studies have shown that between

17.5 and 27.5°C this procedure decreases the rate of exchange of cell K with K^{42} in papillary muscles by a factor of 1.9 (15). Table V indicates no significant changes in cell K and Na contents in muscles cooled to 17–18°C. By contrast, a marked loss of cell K and increases in cell Na, Cl, and water result when the temperature is lowered from 27–28°C to 2–3°C. The changes in ion and water contents at 2–3°C are comparable to those brought about at 27–28°C by 10^{-5} M ouabain, the highest concentration of this inhibitor used. These similarities in the net movements resulting from lowered temperature and from 10^{-5} M ouabain suggest that this cardiac glycoside does not produce its effects through some specific change in passive membrane permeability.

Effects of Removal of NaCl

Table VI presents the results of experiments designed to test the effect of removal of NaCl. When all of the external NaCl is replaced isosmotically by

TABLE V

CELL ION CONTENTS IN COOLED MUSCLES

Temperature	K	Na	Cl
		mmols/kg dry weight	
27–28°C (reference)	343±9	142±18	73±7
17–18°C (9)	340±7	135±19	102±12
2–3°C (10)	98±15	421±23	149±15

sucrose, $[K]_i$ in the control muscles remains at its physiological value of 169 ± 6 mmols/kg cell water (267 ± 7 mmols/kg dry weight). Concomitantly the cell K content and cell volume decrease, reflecting the diffusion of NaCl and KCl out of the cell down their respective transient concentration gradients. The fact that $[K]_i$ does not change is evidence that the lowered ionic strength has not impaired passive membrane permeability to ions. When cells have been equilibrated in NaCl-free solutions, electroneutrality requires that no net movements of K should be possible, the charge on the remaining cellular K being balanced entirely by anions not free to diffuse out of the cell. Table VI shows that the cell K content in the NaCl-free medium does in fact remain unaffected by exposure for 2 hours to 10^{-5} M ouabain or to temperatures of 2–3°C.

Omission of Ca from the NaCl-free bathing solution results in a significant loss of cell K. The changes in permeability to carbohydrates in Ca-poor solution already noted were also found in absence of NaCl, the mannitol space being

72 ± 2 per cent of total water. No external cation is present at a sufficient concentration to exchange with internal K, and the cellular Cl concentration in a medium with only 6.3 mM Cl must be very minute. The observed loss of cellular K therefore indicates that K must be diffusing out of the cell in association with an anion other than Cl. It would appear that the omission of Ca from the medium has increased passive membrane permeability so that an intracellular anion, previously unable to diffuse out of the cell, can now do so. Alternatively, omission of Ca has resulted in the production of an intracellular diffusible anion. For the control muscles in Table VI, calculations based on the restrictions of osmotic equilibrium and electroneutrality (1) yield a total intracellular concentration of indiffusible particles of 208 mM, having an average charge of −0.8. Although the nature of the anion involved is obscure, the possibilities include inorganic phosphate and organic anions.

TABLE VI
ION CONTENTS AND CELL VOLUME
IN SOLUTIONS WITHOUT NaCl

Modification of medium	Cell K	Cell H_2O	Inulin space
	mmols/kg dry weight	*kg/kg dry weight*	*per cent of total water*
NaCl-free control (7)	267±7	1.59±0.07	33±2
10^{-5} M ouabain (7)	274±13	1.44±0.03	35±1
2–3°C (7)	270±13	1.36±0.07	37±2
Ca-poor (8)	185±7	1.41±0.07	39±2

Because of the relatively high molecular weight of sucrose, extracellular sucrose constitutes an appreciable fraction (10 per cent) of the dry weight of muscles in the NaCl-free medium. The absolute cell ion and water contents in Table VI are therefore not directly comparable to those in other tables, though the groups in the table are comparable to one another.

Since the cellular concentrations are not expressed on a dry weight basis, $[K]_i$ is not affected and may be compared with that calculated from the other tables.

DISCUSSION

Active transport of K into the cell coupled to active extrusion of Na from the cell is often inferred from the reciprocal cation movements which result from inhibition by ouabain, lowered temperature, or removal of external K. The present experiments show that external K is necessary for extrusion of Na against the electrochemical gradient, but provide no direct evidence for a

primary effect of cellular or extracellular Na on transport of K. As an alternative to coupled active transport of K and Na, it is instructive to consider the mechanism for the observed ion movements if K in cat heart muscle is passively distributed. A passive distribution for K has already been suggested for frog skeletal muscle by Hodgkin and Horowicz (16). These workers attributed the difference between the K equilibrium potential and the measured transmembrane potential difference to a small but significant passive permeability for Na. Assuming K in the present experiments to be passively transported, the net ion movements after exposure to ouabain or lowered temperature could be produced by a primary inhibition of active Na extrusion. Such inhibition would give rise to a net inward diffusion of NaCl, thereby diminishing the potential difference across the cell membrane. As a result, cell K would no longer be in electrochemical equilibrium and would tend to diffuse outward. According to this view, the site at the external face of the membrane for which ouabain and K appear to compete may be implicated in the active transport of Na.

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