Cat Heart Muscle *in Vitro*

VIII. *Active transport of sodium in papillary muscles*

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ABSTRACT The cells of cat right ventricular papillary muscles were depleted of K and caused to accumulate Na and water by preincubation at 2–3°C. The time courses of changes in cellular ion content and volume and of the resting membrane potential (V_m) were then followed after abrupt rewarming to 27–28°C. At physiological external K concentration ($[K]_o$ = 5.32 mM) recovery of cellular ion and water contents was complete within 30 minutes, the maximal observable rates of K uptake and Na extrusion (Δmmol cell ion/(kg dry weight) (min.)) being 3.4 and 3.6, respectively. The recovery rate was markedly slowed at $[K]_o$ = 1.0 mM. Rewarming caused V_m measured in cells at the muscle surface to recover within from <1 to 9 minutes, but only slight restoration of cellular ion contents (measured in whole muscles) had occurred after 10 minutes. Studies of recovery in NaCl-free sucrose Ringer's solution made it possible to separate the ouabain-insensitive outward diffusion of Na as a salt from a simultaneous ouabain-sensitive Na extrusion which is associated with a net cellular K uptake. A hypothesis consistent with these observations is that rewarming may activate a ouabain-sensitive "electrogenic" mechanism, most probably the net active transport of Na out of the cell, from which net K uptake may then follow passively.

The mechanism by which net movements of K ions are coupled to the extrusion of Na ions from the cell against an electrochemical gradient remains to be clarified in mammalian heart muscle. In the present study we have investigated this problem in cat right ventricular papillary muscle, using a modification of the method applied to frog skeletal muscle by Steinbach (1). Muscle cells were depleted of K and enriched in Na, Cl, and water by preincubation at 2–3°C, then rapidly rewarmed to 27–28°C. The time courses of the net movements of ions and water during recovery from the effects of cooling, as well as of the transmembrane resting potential difference (V_m),

were subsequently followed in solutions containing varying concentrations of K and Na. It was observed that V_m, measured in cells at the surface of the muscle immediately after rewarming, invariably exceeded the K equilibrium potential, (V_K), calculated from chemical analyses of whole muscles. Further experiments in NaCl-free sucrose Ringer's solution support the concept that net uptake of K after rewarming is passive and secondary to the activation of an "electrogenic" extrusion mechanism for Na. The active transport of Na is dependent on the concentrations of intracellular Na and extracellular K, but appears not to depend on the cell volume and the intracellular K concentration, or to require the presence of extracellular Na.

METHODS

Recovery of Cooled Muscles after Rewarming

The experiments were designed to measure the rate of net extrusion of Na and net uptake of K and to correlate these net ion movements with changes in the resting transmembrane potential difference (V_m). For this purpose, papillary muscles from cats anesthetized with pentobarbital were dissected in the chamber of Page and Solomon (2). The muscles were then transferred to a vessel for incubation with small volumes of radioactive solution (3). In these vessels they were preequilibrated for 1 hour in normal Ringer's solution labeled with H^3-mannitol, C^{14}-inulin, and, in selected experiments, Cl^{36}. The temperature of the bath was then lowered to 2–3°C for the desired period (1 or 2 hours). At the end of this period the muscles were either removed for analysis or rewarmed to 27–28°C and incubated at that temperature, for intervals of from 10 to 90 minutes prior to removal. Subsequently the muscles were analyzed for their contents of inulin, mannitol, Cl^{36}, K, Na, and water as described by Page (3).

Rapid changes in the temperature of the incubation fluid were made by adding ice or warm water to a bath, from which water was continuously circulated by a pump to the water jackets around the incubation vessels. The temperature change from 2–3°C to 27–28°C required approximately 5 minutes in the first series of experiments. Subsequently, we learned how to accomplish this change in 1.2–1.5 minutes, and the experiments correlating ion movement with V_m were carried out in this way.

In two series of experiments the K concentration of the solution in which muscles were rewarmed was lowered to 2.5 and 1.0 mM, respectively. For these experiments the 5.32 mM K medium in the cooled bath was changed to the lower K concentration before rewarming, to facilitate equilibration of the extracellular compartment with the K concentration of the bath.

Two series of experiments were designed to test recovery of cell K in NaCl-free sucrose Ringer's. To this end, muscles were immediately transferred from the dissection chamber to cold (2–3°C) phosphate-buffered Ringer's solution with the physiological (178.5 mM) external Na concentration. After the cells had taken up Na, Cl, and water and lost K for 90 minutes, the extracellular compartment was depleted of Na and Cl by changing the bathing medium to an isosmolar, NaCl-free sucrose solution containing the same (5.32 mM) external K concentration. Muscles were kept in

this bath at 2–3°C for a second 90 minute interval, the solution being labeled with H^3-mannitol and C^{14}-inulin for measurement of extracellular volume. At the end of this period one muscle was removed for analysis, the other rapidly rewarmed to 27–28°C and equilibrated in the NaCl-free medium for 45 minutes. The identical procedure was followed in a second series of experiments, which differed only in the addition of 10^{-4} M ouabain to both the NaCl-containing and NaCl-free solutions.

Selected muscles, instead of being transferred from the dissection chamber to the incubation vessels, were placed immediately in a lucite box (internal dimensions 12.5 × 11.0 × 6.3 cm) filled with cold Ringer's solution. In this box they were stretched to approximately their *in situ* length by suspending them with silk thread between perforated pegs protruding from the floor of the chamber. Fluid in this chamber was continuously oxygenated, stirred with a magnetic stirrer, and kept at 3–4°C in a refrigerated room for 2 hours. At the end of this period, the muscle was transferred to a chamber for microelectrode measurements (4) by threading the silk sutures (by which the ends of the muscle had been attached to the pegs in the box) into perforations drilled through the floor of the microelectrode chamber. This chamber was perfused at a rate of 32 ml/minute with Ringer's solution at 4–6°C. The temperature of the solution was monitored to ±0.2°C with a thermistor placed near the muscle. The muscle could be rewarmed from 4–6°C to 27–28°C within 20 seconds by a double stopcock arrangement. This arrangement permitted selection of perfusing solution from either of two water-jacketed reservoirs, the temperatures of which were maintained at 29 and 2°C, respectively. (The differences between the temperatures of the reservoirs and muscle chamber reflect heat exchanges in the connecting plastic tubing.)

The electrical recording system was identical to that used by Page (4), except that potential differences were read directly on the electrometer (Keithley, model 200 B). Muscle diameters were measured as previously described (3).

Solutions

The basic solution was a bicarbonate-buffered medium (2) bubbled with 5 per cent CO_2, 95 per cent O_2, the composition of which was, in mM, K 5.32, Na 178.5, Cl 163.1, Ca 1.40, Mg 0.56, HCO_3 22.0, HPO_4 0.59, H_2PO_4 1.45, and dextrose 5.5. Solutions with K concentrations of 2.5 and 1.0 mM were obtained by a replacement of KCl by NaCl. An isosmolar phosphate-buffered solution with 10 mM K (bubbled with 100 per cent O_2) was used for the one series of experiments requiring both microelectrode studies and chemical analyses. This solution contained enough K to prevent the violent contractions invariably observed in muscles which are rapidly rewarmed in media with a physiological (5.32 mM) K concentration. Such contractions have the undesirable effect of breaking microelectrodes or displacing them from the cells. Moreover, it was not feasible to bubble with CO_2 directly within the muscle chamber; for experiments involving relatively large temperature changes it therefore seemed preferable to use a medium with a buffering capacity not dependent for the maintenance of the pH on the partial pressure of CO_2. The composition of the solution used was, in mM, K 10.0, Na 178.7, Ca 1.40, Mg 0.56, Cl 189.8, HPO_4 1.295, H_2PO_4 0.205, dextrose 5.5. A third medium buffered with phosphate, in which all of the

external NaCl was replaced by sucrose, was isosmolal with physiological cat Ringer's solution as measured by the freezing point depression using the Fiske osmometer (model G-62). Its composition was, in mM, K 5.32, H_2PO_4 0.205, HPO_4 1.295, Ca 1.40, Mg 0.56, Cl 3.92, dextrose 5.5, and sucrose 295 (total osmolality 345 mOsM/kg water).

RESULTS[1]

Recovery during Rewarming in HCO_3-Ringer's Solutions

Table I and Fig. 1 show the rate at which the physiological cellular ion contents and concentrations were reestablished when muscles were rewarmed from 2–3°C to 27–28°C in HCO_3-buffered Ringer's solution with 5.32 mM K. Straight lines have been drawn connecting the experimental values for the cellular cation concentrations at 15 and 30 minutes after rewarming, the interval during which the rate of recovery measured by chemical analysis appears to be maximal (Fig. 1). The slope of this line is a mean value. If an inflection in the curve occurs between the 15 and 30 minute points, the maximum slope might greatly exceed this mean. For each muscle the cell K and Na contents per kg dry weight were calculated by multiplying the cellular ion concentration by the cell water content per kg dry weight (Table I). The rates of cell K uptake or Na extrusion [Δ(mmol cell ion)/(kg dry weight) (minute)], obtained in this way for the 15 to 30 minute interval, are likewise mean (and

TABLE I

EFFECT OF REWARMING ON CELL ION CONTENT* (BICARBONATE BUFFER)

$[K]_o$	Duration of recovery period‡ at 27°C	Cell ion content			Cell water content	No. of experiments
		K	Na	Cl		
mM	*(min.)*	*mmols intracellular ion / kg dry weight*			*(kg/kg dry weight)*	
5.32	Not rewarmed	290±9	207±7	83±5	2.45±0.06	11
	15	300±17	156±23	88±4	2.29±0.05	8
	30	356±12	104±7	79±4	2.33±0.04	7
	45	370±8	102±8	89±5	2.19±0.04	9
	90	346±13	123±10	97±5	2.16±0.04	9
2.5	90	359±9	126±4	96±9	2.29±0.03	8
1.0	90	314±12	154±14	109±11	2.32±0.06	8

* Muscles were incubated 1 hour at 2–3°C.

‡ Exclusive of time required for rewarming.

[1] Results in tables, text, and Fig. 1 are expressed as mean ± standard error. Significance of differences is calculated with the Student *t*-test for series of less than 30 measurements, and is reported "significant" if $P < 0.01$. Intracellular ion concentrations, cellular ion contents, and cell volume are calculated based on inulin spaces, unless otherwise indicated.

not maximal) estimates of the net cation fluxes during this interval. The figure for the net K influx was 3.7, when calculated using either inulin or mannitol to measure extracellular volume. The corresponding figures for the net rate of Na extrusion were 3.4 (based on the inulin space) and 3.6 (based on the mannitol space). Accordingly, one K ion is taken up by the cell for

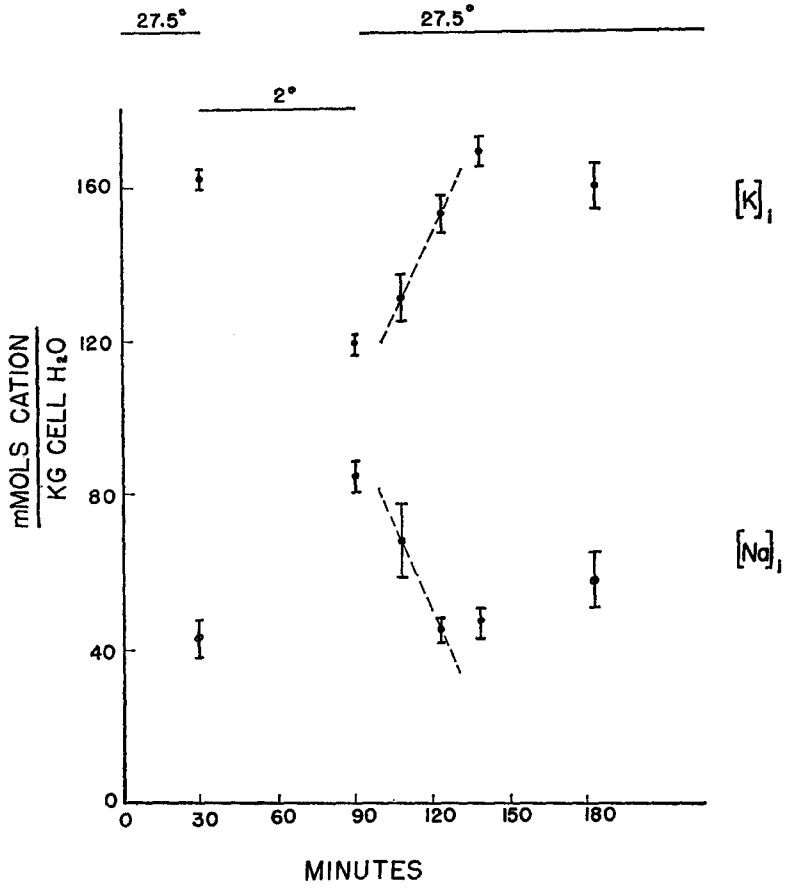

FIGURE 1. Effects of cooling and rewarming on intracellular concentrations of K (upper plot) and Na (lower plot).

each Na ion expelled during the interval between 15 and 30 minutes after rewarming. Under these non-steady state conditions, the net flux for K and Na approximates the steady state exchange rate for K at 27–28°C as determined by Goerke and Page (6).

After 30 minutes at 27–28°C the cell contents of K and Na have returned to values physiological for papillary muscles (2, 6). By contrast, when the K concentration of the solution in which muscles are rewarmed is reduced to 1 mM, recovery is less complete after 90 minutes than after 30 minutes in 5.32

mM K. At the intermediate external K concentration of 2.5 mM, cell K content is completely restored at 90 minutes. A significant extrusion of cell Na does occur even in 1 mM K solution, the cell Na content being lowered by 53 mmols/kg dry weight after 90 minutes. (The observed recovery rates in 1 mM and 2.5 mM K solutions may be unduly high. In spite of a brief period of exposure to the lowered K concentration at the end of the interval in the cold, it seems likely that at the start of rewarming the local extracellular K concentrations near the axis of the muscle cylinder may have been higher than the K concentration of the medium. On rewarming, such localized increases in K concentration would have tended to increase the initial net ion movements.)

The failure of the cell Cl content to decrease on rewarming is noteworthy and remains unexplained. This finding is not an artifact resulting from the possibly incomplete equilibration of the extracellular space with inulin (3), since the cell Cl content after 45 minutes calculated on the basis of the mannitol space, determined simultaneously with the inulin space, also does not diminish. Cell Cl contents (mmols cell Cl/kg dry weight) derived from mannitol space measurements after 0, 15, 30, 45, and 90 minutes of rewarming were, respectively, 34 ± 4, 30 ± 6, 31 ± 3, 39 ± 4, and 18 ± 5, the 90 minute value being the only figure significantly lower than that for 2–3°C. Thus the significant diminution in cell water content that has occurred at 45 minutes cannot be attributed to a net outward diffusion of cellular Cl as NaCl, accompanied by the osmotic equivalent of water.

Comparison of Recovery of Cellular Ion Contents and Concentrations with Recovery of V_m

Transient, vigorous, spontaneous contractions immediately after rewarming were observed uniformly in solutions with physiological (5.32 mM) or lower external K concentrations, in spite of the low initial cellular K concentrations of the cooled muscles. To avoid the resulting interference with membrane potential measurements, experiments were carried out in partially depolarizing solutions with 10 mM K. At this higher external K concentration, brief (duration < 2 minutes) spontaneous contractions on rewarming occurred only four times in twenty experiments. From the thermal diffusivity for water, the time required for thermal equilibration of a cylindrical tissue of the size used (mean muscle diameter 0.71 to 0.73 mm) can be calculated to be negligibly small relative to the time scale of the observed membrane potential change. Moreover, recovery of V_m after rewarming was observed to be complete in less than 10 minutes. It was therefore decided to measure the recovery of cellular ion concentrations after 10 minutes at 27–28°C (not counting 1.2 to 1.5 minutes required for warming the bathing medium). Since restoration of V_m is already complete some minutes before the end of this interval, chemical analysis after 10 minutes should show to what extent this recovery is correlated with net movements of K and Na.

Fig. 2 and Table II present the time course of V_m when muscles, pre-incubated for 2 hours at 4°C in a medium with 10 mM K, are suddenly warmed to 27–28°C. The mean value of V_m (in millivolts) in the cold was 25.6 ± 0.7 (cellular solution negative with respect to the extracellular solution), which may be compared with a figure of 61.3 ± 0.5 obtained by averaging the approximately constant values of V_m between 10 and 85 minutes after rewarming. Fig. 2 illustrates the striking speed with which V_m responded to a

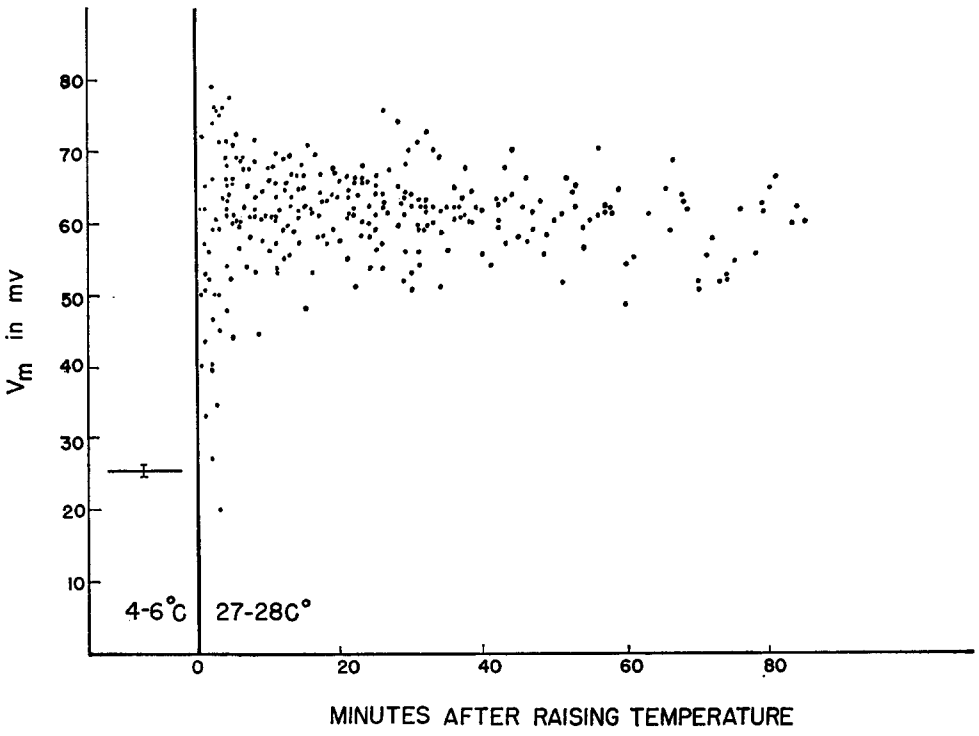

FIGURE 2. Effect of sudden rewarming on V_m in phosphate-buffered solution with 10 mM K. Period of rewarming is indicated by a vertical bar. Values of V_m prior to rewarming are given as mean ± standard error.

rise in the temperature of the perfusing solution. Recovery to a stable level (as high as or transiently higher than 61 mv) was observed to occur within less than 1 minute in four experiments, within less than 4 minutes in seven experiments, and within 9 minutes in one experiment. Recovery may have occurred earlier than these figures indicate, since it was not always technically possible to penetrate the cells successfully within the first minute after rewarming. In two experiments, in which the electrode remained in the cell during the change from cold to warm solutions, V_m was observed to have recovered or overshot even before the temperature gauge indicated a return to 27–28°C. In one of these two experiments the rise of V_m appeared to be complete in less

than 15 seconds, in the other, so rapidly that the time required could not be accurately estimated. Total failure of V_m to respond to rewarming was encountered in one muscle (not included in Fig. 2 or Table II). Control measurements with the microelectrode in the bath established that no significant signal artifact was produced on changing from cold solution to the medium at 27–28°C.

Table II gives the results for muscles analyzed after 10 minutes of recovery. It is apparent that values of $[K]_i$ and $[Na]_i$ have not yet been restored to physiological levels long after V_m has returned to normal. As a result, while V_m changes from 25.6 to 61.3 mv, the K equilibrium potential, V_K, given by $(RT/F)\ln([K]_i/[K]_o)$, changes only from 41 to 55 mv. The net K uptake and net Na extrusion during the 10 minute period were averaged for each muscle

TABLE II*

CELLULAR CATION CONCENTRATIONS AND V_m AFTER 10 MINUTES OF REWARMING (PHOSPHATE BUFFER)

	$[K]_i$	$[Na]_i$	V_K	V_m
	mmols/kg cell H_2O		*mv*	*mv*
2 hrs. at 2–3°C	58±4	193±14	41±2 (12)	25.6±0.7 (138)
Recovery for 10 min. at 27.5°C	85±6	131±10	55±2 (12)	61.3±0.5 (196)

* Results of experiments from twelve cat hearts from which three papillary muscles were obtained (one each for chemical analysis after cold incubation, for chemical analysis after rewarming, and for measurement of V_m). Figures in parentheses give number of measurements of V_m. Muscle diameters (mm) were 0.71 ± 0.07 and 0.73 ± 0.06 for the cold and rewarmed muscles, respectively. Cell water contents (kg cell water/kg dry weight) were 2.3 ± 0.1 and 2.4 ± 0.1 for cold and rewarmed muscles, the corresponding values for inulin spaces (per cent of total muscle water) being 25 ± 1 and 27 ± 2 respectively.

pair, yielding (in mmols cell ion/kg dry weight) a net K uptake of 75 ± 9 and net Na loss of 144 ± 28. Although a discrepancy between Na extrusion and K uptake is suggested by their mean values, a significant difference between these two figures cannot be established statistically because of the large dispersion of the data for Na ($P > 0.05$). Cell H_2O content did not change, whether determined with inulin (Table II) or mannitol to measure the extracellular space.

In exploratory experiments, 10^{-4} M or 10^{-5} M ouabain was incorporated into the medium during both the equilibration in the cold and the rewarming period. 10^{-4} M ouabain completely prevented the recovery of V_m when the temperature was raised to 27–28°C. V_m rose transiently to values near 60 mv within 1 minute after rewarming in 10^{-5} M ouabain. These values were not, however, maintained as in the absence of ouabain. Within 5 minutes after rewarming, V_m began a progressive decline, approaching its control (cold) value after 60 to 80 minutes at 27–28°C.

Recovery in NaCl-Free Solution

Table III summarizes the recovery on rewarming in K-depleted muscles with high cellular contents of Na and Cl when the rewarming is carried out under conditions in which the extracellular NaCl has been replaced with sucrose by preequilibration in the cold with NaCl-free sucrose Ringer's solution. Papillary muscles do not well tolerate prolonged incubation and rapid changes of temperature in solutions of such low ionic strength, which may damage a fraction of the cells, rendering them non-specifically permeable to ions and to sucrose. Damage to a fraction of the cells is the most likely explanation for the comparatively low total ion content/kg dry weight and for the large mannitol space of 57 ± 5 per cent of total water in muscles rewarmed in NaCl-free

TABLE III*

EFFECTS OF REWARMING TO 27–28°C IN NaCl-FREE SOLUTION WITH AND WITHOUT OUABAIN

Experiment	Cell K	Cell Na	Cell water	Inulin space
	mmol cell ion/kg dry weight		*kg/kg dry weight*	*per cent total water*
2–3°C (7)	129	199	2.3	19
	±18	±26	±0.1	±2
Rewarmed (7)	196	35	1.5	33
	±12	±7	±0.1	±3
2–3°C, 10^{-4} M ouabain (6)	136	208	2.37	17
	±21	±23	±0.07	±2
Rewarmed, 10^{-4} M ouabain (7)	88	71	1.51	34
	±4	±8	±0.07	±3

* Figures in parentheses give number of experiments. Weight of sucrose content of extracellular space has been subtracted from total dry weight and extracellular water content derived with a correction for the change in specific gravity due to sucrose. The extracellular Na concentration is assumed to be zero in calculating the cellular Na content.

medium. The critical questions to be decided experimentally are, however, whether in the remaining cells a net loss of cell Na and an uptake of K can occur in absence of external Na and Cl, and whether any fraction of these net cation movements is ouabain-sensitive. Table III shows that rewarming under these conditions does in fact bring about a significant net cellular uptake of K and net cellular losses of Na and water. These net ion and water movements cause the calculated cellular K concentration (mmols/kg cell water) to rise from 56 ± 9 to 124 ± 9, while the cellular Na concentration falls from 91 ± 12 to 22 ± 5. The net cellular uptake of K and loss of Na were determined for muscle pairs from the same heart and averaged. The figures so obtained (Δmmols cell ion/kg sucrose-free dry weight) were +(74 ± 10) for K and −(164 ± 26) for Na. These values, showing that the net loss of Na significantly exceeds the net uptake of K, reflect the fact that half or

more of the reduction in cell Na after rewarming occurs as NaCl or as Na lost with normally fixed cellular anion, the remainder being accounted for by an exchange of cellular Na for extracellular K.

The effects of addition of ouabain were tested at a ouabain concentration (10^{-4} M) which completely inhibits recovery of the membrane potential on rewarming (see previous section). It is apparent from Table III that ouabain prevents the net cellular uptake of K from NaCl-free solution, but does not inhibit that fraction of net cellular loss of Na associated with a marked fall in cellular water content. It would appear that NaCl and its osmotic equivalent of water, taken up by the cells at 2–3°C in NaCl-containing medium, remain trapped within the cells when external NaCl is reduced to zero in the cold. Alternatively or in addition, an anion (*e.g.*, inorganic or organic phosphate) which cannot diffuse out of the cell at a significant rate in the cold, is produced within the cell, where it serves as the osmotically active counterion to a part of the Na taken up by the cell. When the temperature is suddenly raised, this fraction of cellular Na, accompanied by its anion and osmotic equivalent of water, diffuses passively out of the cells. The cellular Na content remaining after rewarming is, however, significantly higher in the ouabain-treated muscles, indicating that ouabain has interfered with that component of the net cellular Na extrusion which normally involves a direct exchange for extracellular K.

After the usual 1 hour preincubation at 27.5°C in phosphate-buffered medium containing Ca, paired muscles were immersed at the same temperature for 30 minutes in a solution from which Ca had been omitted. They were then equilibrated at 2–3°C for 2 hours, one each of the paired muscles being removed for analysis before and after rewarming for 45 minutes. The inulin spaces of the rewarmed muscles were large (55 ± 8 per cent of total water, range 31 to 87 per cent), suggesting that the cells had become partially permeable to this carbohydrate. Calculation of intracellular ion concentrations based on inulin spaces thus becomes uncertain and the results are therefore given in terms of the total ion contents of the muscle, expressed as mmols ion/kg dry weight. The tissue K content in these units rose from 129 ± 8 after 2 hours in the cold to 325 ± 2 after 45 minutes at 27–28°C. The corresponding figures for Na are 67 ± 9 at 2–3°C and 37 ± 5 at 27–28°C (11 paired muscles). These results indicate that extrusion of Na and uptake of K can take place at the very low concentrations of extracellular ionized Ca which must have prevailed after prolonged exposure to Ca-free solutions.

DISCUSSION

The Discrepancy between V_m and V_K

When papillary muscles are suddenly rewarmed, V_m, defined as in (6) and measured in surface cells, exceeds V_K, calculated from the cellular K content

of the muscle as a whole. This apparent discrepancy between V_m and V_K during recovery is the reverse of that prevailing prior to rewarming (Table II) as well as of that previously observed in the steady state (4). Both in the steady state at 27–28°C and in the K-depleted, Na-enriched state at 2–6°C (Table II) $V_m < V_K$ at physiological and higher external K concentrations. The reversal of the usual relationship between V_m and V_K during recovery suggests at least two alternative interpretations of the nature of active ion transport in heart muscle. Before discussing these alternatives it is necessary to consider whether the cellular K concentration measured in the rewarmed muscle as a whole faithfully reflects that of surface cells, and whether the K concentration bathing the muscle surface corresponds during rewarming to the extracellular K concentration bathing cells in the interior of the muscle.

If the cellular K content measured chemically in whole papillary muscles after 10 minutes of rewarming does correspond to the K content of the cells at the muscle surface, V_m must in fact exceed V_K for all the cells in the muscle. In this case V_m would appear to be determined by factors other than the ratio of cellular to extracellular K concentrations. The observation that the rapid rise in V_m on rewarming is markedly ouabain-sensitive argues strongly against a primary change in the passive permeability of the membrane to ions. Ouabain sensitivity might, however, be anticipated if the primary effect of a sudden raising of the temperature is an abrupt initiation of an electrogenic active transport of Na out of the cell. This interpretation would be consistent with our previous suggestion of a passive distribution for K ions in heart muscle (5). After V_m is suddenly raised by the activation of an electrogenic Na pump, K ions would tend to redistribute themselves according to the new V_m; the rate of cellular K uptake at any instant would then be a function of the membrane permeability for K and Cl, of the prevailing K concentration gradient, and of the potential difference across the cell membrane.

A sudden rise in temperature may, however, bring about a cellular uptake of K sufficiently rapid to deplete the extracellular concentration of K at surfaces of cells located within the interior of the muscle. For such a depletion to occur, the net flux of K into the cells must be so large that net diffusion of K from the solution perfusing the muscle would be too slow to maintain a uniform extracellular K concentration. Under these conditions, a concentration gradient for extracellular K would exist, ranging from the lowest extracellular K concentrations at the axis of a cylindrical muscle to a maximum equal to the K concentration of the bath at the muscle surface. The observations of Page, Goerke, and Storm (5) and of the present paper clearly indicate that Na extrusion and K uptake are inhibited at external K concentrations of 1 mM or lower. A rapid K uptake by the cells would therefore inhibit active transport in the muscle interior without slowing the K uptake of surface cells, assuming extracellular K diffusion is not rate-limiting for surface cells. As a

result, the extrusion of Na by and the flux of K into the surface cells (on which microelectrode measurements are most conveniently made) would greatly exceed the net ion movements disclosed by chemical analysis on whole muscles.

The occurrence of depletion of extracellular K in the muscle interior during rewarming is supported by the following experiment: a microelectrode inserted into a cell in the interior of the muscle does not record the same rapid recovery of V_m observed in cells at the muscle surface. V_m measured in three experiments 0.45 mm below the muscle surface had not recovered to the value determined in surface cells of the same muscle until 17, 21.5, and 16.5 minutes, respectively, after rewarming. Thus, significant lowering of the extracellular K concentration in the muscle interior seems highly probable.

Nevertheless, an electrogenic mechanism activated by rewarming remains a useful hypothesis by virtue of the following considerations: If the recovery of V_m within 1 minute after raising the temperature to 27–28°C does in fact reflect the restoration of the contents of K and Na in *surface cells* to their physiological values, it is possible to approximate the mean net cellular ion fluxes that must have occurred in these surface cells in order to effect such a restoration. For this purpose the physiological cell K and Na contents (mmols cell ion/kg dry weight) may be taken as 356 and 104, respectively, corresponding to the figures recorded after 30 minutes' recovery in Table I. From the results of chemical analyses on cooled muscles (Table II), the mean K influx is calculated as 223 mmol/(kg dry weight) (minute), and the mean Na efflux as 343 mmol/(kg dry weight) (minute). The net K uptake during 1 minute so computed would be about 45 times greater than the steady state K exchange of papillary muscles at 27–28°C derived for an external K concentration of 10 mM from Fig. 6 of the study by Goerke and Page (6). When recovery took place in less than 15 seconds, as observed in two experiments, this calculation yielded a rate of K uptake more than 180 times that for steady state exchange. So rapid a rate of net K uptake, while not inconceivable, seems on *a priori* grounds less likely than the alternative of an electrogenic net cation extrusion.

It is also possible that the net K uptake by surface cells on rewarming takes place at a rate comparable to that for steady state K exchange, but involves only a very small fraction of the K content of surface cells; *i.e.*, a fraction $<1/180$ of the total cellular K content in the steady state. This interpretation would imply that the K concentrations of relevance to the membrane potential on rewarming are those at a localized cell surface region where the behavior of K ions differs from that in the bulk of the cytoplasmic solution. In order to reconcile such a mechanism with the observation that all of cell K exchanges at a single rate (6), it would be necessary to assume a model for the relation of K ions in the extracellular space, surface layer, and cytoplasmic bulk solu-

tions (7). Localization of the relevant K movements in a cell surface region would reduce the magnitude of the net K movement, but would not obviate the need for an electrogenic extrusion of Na (or other ouabain-sensitive mechanism) causing the surface layer to take up K on rewarming. In this connection, it is of interest that Hodgkin and Horowicz, who found that all of cell K in single frog skeletal muscle cells exchanges at a single rate (8), adduced evidence for retention of K in a special region estimated as $\frac{1}{500}$ to $\frac{1}{200}$ of the fiber (9). Adrian and Freygang have also presented observations interpreted in terms of a K-permeable intermediate extracellular compartment of restricted volume (10). The possible involvement of a small fraction of cell K in the present experiments on papillary muscle appears at first to be inconsistent with the observations indicating reduction of the extracellular K concentration in the muscle interior during rewarming. The latter observations suggest that the net uptake of K by the cells is of sufficient magnitude to deplete the K concentration at the cell surface. Depletion of K ion in the interior of the muscle might, however, occur during rewarming if, as suggested by Adrian and Freygang (10) for frog skeletal muscle, net movements of K into the heart muscle cell originate in a relatively small extracellular subcompartment. A critical decrease in the K concentration of such a subcompartment would arise if diffusion of K ions into this space from the tissue interspaces were slow relative to the net movement of K from the subcompartment into the cells.

Results of measurements of steady state K exchange (11) do not support a third alternative possibility, that of a carrier-mediated "electroneutral" mechanism for K uptake. In such a mechanism one Na ion is extruded for each K ion taken up by the cell, both K and Na combining successively with the same site on the carrier molecule (12). Against this interpretation is the observation that steady state K exchange is independent of the presence of Na over a wide range of temperatures (11). The exchange is, moreover, ouabain-insensitive in absence of NaCl. It is therefore improbable that K and Na can utilize a single site on the same carrier. Instead, K at the external face of the cell membrane is required for Na extrusion; the unwarranted inference that movements of Na and K are coupled by utilization of the same carrier derives from the net loss of cell K which ensues secondarily when Na extrusion is inhibited in K-free media.

Interpretation of Observations in NaCl-Free Solution

The observations in NaCl-free solution suggest that a ouabain-sensitive net cellular uptake of K can occur in the virtual absence of external Na and Cl. The results further indicate that this exchange of cellular Na for extracellular K is separable from the outward diffusion of Na as a salt (NaCl or Na plus a counterion of cellular origin) with its attendant reduction in cell water con-

tent. This separation may be achieved because the outward diffusion of Na as an electroneutral salt, unlike the carrier-mediated Na extrusion, is not inhibited by ouabain. The sensitivity to ouabain of net K uptake and of extrusion of Na as Na ion also rules against the possibility that the K uptake is secondary to a diffusion potential generated during net outward diffusion of Na as a salt. The persistence of these ouabain-sensitive net cation movements in NaCl-free solution would also seem to exclude an active transport of Cl into the cell as the primary event on rewarming.

At the very low (3.92 mM) external Cl concentration prevailing in the NaCl-free medium virtually all of the cellular Na which had originally entered in direct exchange for K passively lost by the cell must have been present as the counterion to intracellular anions not normally free to diffuse out of the cell. On sudden rewarming the exchange of this fraction of cell Na for extracellular K could occur in two ways. One possibility is a cation exchange *via* the "passive channel," *e.g. via* an aqueous electrolyte solution within the interstices of the cell membrane whose passive ion permeability has been altered by the temperature change. A process of this type seems unlikely, in view of the ouabain-sensitivity of the net cellular K uptake. A more satisfactory alternative is a primary electrogenic extrusion of Na *via* an ouabain-sensitive carrier mechanism, giving rise secondarily to a cellular uptake of K through the passive channel.

Page, Goerke, and Storm (5) have presented evidence that ouabain may inhibit active Na extrusion in heart muscle by competing with K ions for a site located at the external face of the cell membrane. This effect of ouabain could be simulated by exposure to K-free solutions which reduce the K concentration outside the cell to zero. According to this view, the effect of ouabain in the present experiments involves a displacement of K at the external surface of the membrane, thereby inhibiting an electrogenic, carrier-mediated extrusion of Na. Since extracellular diffusion of Na in heart muscle is rapid (13), the concentration of Na at the external surface of the cell membrane must be very low during rewarming, when the only source of extracellular Na is Na diffusing out of the cells. It would therefore appear that, as in erythrocytes (14), active transport of Na in heart muscle depends on the concentration of external K and intracellular Na, but not on the concentrations of intracellular K or on the presence of external Na. In this connection it is of interest that a particulate membrane fraction with ouabain-sensitive Na- and K-stimulated adenosinetriphosphatase activity has been isolated from mammalian heart muscle by a number of investigators (15–18). If this enzymatic activity is indeed an enzymatic analogue of active transport of Na in intact heart muscle, the results of the present study suggest that the Na-stimulated site on the enzyme or structure should differ from the site stimulated by K and inhibited by ouabain.

The present results, suggesting an electrogenic cation extrusion, do not specifically limit this mechanism to the Na ion. A primary active transport out of the cell of a different cation, for example, calcium, could likewise account for the experimental observations.

Electrophysiological Implications of Electrogenic Na Extrusion

The observations of the present paper suggest that activation by rewarming of an electrogenic mechanism, presumably the active extrusion of Na, may produce a rapid repolarization. It seems probable that the rate of repolarization may greatly exceed that detectable with the time resolution of the rewarming system and of the instrumentation for measuring V_m employed here. If repolarization by activation of electrogenic Na extrusion occurs under physiological conditions, this process may be of considerable importance in fixing the level of the membrane potential, in modifying it during the repolarization phase of the action potential, or in affecting excitability during electrical diastole. In future studies it will therefore be of interest to look for physiological mechanisms for activating and inhibiting Na extrusion in heart muscle; *i.e.*, for physiological analogues for the rewarming and cooling procedures used by us.

Spontaneous Contractions in Muscles with Altered Cellular Ion Concentrations

The repetitive muscle contractions immediately on rewarming in 5.32 mM K solutions merit comment in the light of the low mean cellular K concentrations and high mean cellular Na concentrations of the muscle as a whole prevailing at this time (Table II). This observation suggests that action potentials in ventricular muscle cells can occur even if the cytoplasmic solution has become markedly depleted of K and enriched in Na. Moreover, such action potentials would have to be elicited in the presence of a cellular Na concentration which is not statistically different from the extracellular Na concentration. (Although action potentials were not directly recorded, the fact that contractions were repetitive and abolished by depolarizing solutions makes it highly probable that these contractions were associated with action potentials.)

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