# Cat Heart Muscle *in Vitro*

## VII. *The temperature dependence of steady state K exchange in presence and absence of NaCl*

ERNEST PAGE

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ABSTRACT In quiescent cat papillary muscles $J_K$, the rate of exchange of cellular K with $K^{42}$ in the steady state, has been measured in the presence and absence of NaCl over a wide range of temperatures. $J_K$ was found to be independent of the presence of external NaCl under the steady state conditions investigated. The Arrhenius plot for K exchange was linear over a range of temperatures from 2.5 to 37.5°C in the absence of NaCl, and from 17.5 to 37.5°C in the presence of NaCl. The corresponding apparent activation energies were, respectively, 10,200 and 8,800 calories/mole. $J_K$ in the absence of NaCl was not affected by $10^{-5}$ M ouabain. These results are consistent with a passive distribution for the K of heart muscle cells. The observations suggest that a carrier-mediated forced exchange of K for Na does not occur during the steady state in mammalian heart muscle.

Evidence for coupling between movements of K and Na in cat heart muscle has been obtained under conditions in which the physiological steady state is disturbed by inhibition of active cation transport (1). In the present paper, $J_K$, the rate of exchange of cellular K with $K^{42}$ in the steady state, has been measured in quiescent cat papillary muscles in the presence and total absence of NaCl over a wide range of temperatures. The results show that $J_K$ is independent of the presence of external Na under the steady state conditions investigated. The Arrhenius plot for K exchange in the absence of NaCl has been found to be linear over a range of temperatures from 2.5–37.5°C. These observations are consistent with a passive distribution for the K of heart muscle cells. They suggest that a carrier-mediated forced exchange of K for Na of the type proposed by Ussing (2) for the muscle cell does not occur during the steady state in mammalian heart muscle.

## METHODS

Early in the course of the experiments described in the preceding paper (3) it was found that the cellular K uptake at physiological external K concentrations was linear for more than 30 minutes. Such linearity suggested the possibility of approximating the (unidirectional) K influx by a single measurement of the radioactivity of a muscle removed after a fixed period of equilibration with $K^{42}$. The duration of equilibration was chotsen to be sufficiently long for extracellular K to equilibrate with $K^{42}$ from the bath, ye short enough to fall within the period of linear cellular $K^{42}$ uptake. The time required for equilibration of the extracellular space by diffusion may be approximated by the graphical method of Hill (4), provided the diameter of the muscle cylinder and an estimate of the diffusion coefficient are available. If the diffusion coefficient for K at 27.5°C is one-half of the free solution value (5), it can be calculated that extracellular equilibration should be more than 99 per cent complete in less than 2 minutes for muscles having the average diameter of the papillary muscles used here. Accordingly, the incubation times selected to assure complete extracellular equilibration were 8 min utes at 37°C, 15 minutes at 27.5°C, 25 minutes at 17.5°C, and 30 minutes at 2–3°C.

It was desirable to measure K exchange simultaneously in two muscles from the same heart, as well as in muscles too small to be satisfactorily counted with the end-window counter described in the preceding paper (3). For this purpose quiescent cat right ventricular papillary muscles or trabeculae carneae were immersed in solutions containing $K^{42}$, using the vessels designed for incubation in small volumes of bathing solution described by Page (6). All muscles were preequilibrated for 1 hour at 27.5°C in non-radioactive solution containing NaCl, then incubated for the desired length of time and at the desired temperature in the radioactive medium. At the end of the $K^{42}$ influx period, muscles were removed from the bath, blotted, weighed, dried, reweighed, assayed for $\gamma$-radiation in a well-type scintillation counter, extracted with redistilled 0.1 N $HNO_3$, and the extract analyzed for K content as previously described (7). For experiments in NaCl-free solutions, a 30 minute rinse in non-radioactive NaCl-free medium preceded the $K^{42}$ influx measurement to allow NaCl to diffuse out of the muscle. The trace amount of Na remaining after this period was too small to be estimated by the flame photometric assay used.

To calculate the cellular K exchange, the counts per minute attributed to the radioactivity of the extracellular compartment were subtracted from the total muscle radioactivity. This correction may be appreciable when equilibration with $K^{42}$ has been relatively brief. For this purpose the extracellular space was taken as the inulin space, as previously determined (1, 7), and the extracellular K concentration was assumed equal to that of the incubation medium. The fractional equilibration was then calculated from the quotient of cellular and bath specific radioactivities, and the exchange constant, $k$, defined as in (3), obtained by dividing by the number of minutes equilibrated with $K^{42}$.

### *Solutions*

The basic solution was a phosphate-buffered medium, pH 7.2, bubbled with 100 per cent $O_2$, isosmolar with the physiological (bicarbonate-buffered) medium used in the

previous paper. Its composition (in mM) was K 5.32, Na 178.5, Cl 179.6, Ca 1.40, Mg 0.56, $H_2PO_4$ 0.205, $HPO_4$ 1.295, and dextrose 5.5. For experiments in NaCl-free sucrose, a mole for mole equivalent substitution of sucrose for NaCl gave a medium of the following composition (in mM): K 5.32, $H_2PO_4$ 0.205, $HPO_4$ 1.295, Ca 1.40, Mg 0.56, Cl 6.3, dextrose 5.5, and sucrose 357.

TABLE I

K EXCHANGE IN PRESENCE AND ABSENCE OF NaCl*

| Temperature | $J_K$ | $k$ | Muscle diameter | $[K]_o$ | No. of experiments |
|---|---|---|---|---|---|
| °C | *mmols cell K exchanged* / *(kg dry weight) (min.)* | *(min.)*$^{-1}$ | *mm* | *mM* | |
| | | NaCl 178.6 mM | | | |
| 17.5 | 2.7 | 0.0079 | 0.69 | 5.39 | 10 |
| | ±0.3 | ±0.0008 | ±0.08 | ±0.02 | |
| 27.5 | 4.8 | 0.013 | 0.85 | 5.29 | 15 |
| | ±0.4 | ±0.001 | ±0.07 | ±0.04 | |
| 37.5 | 7.6 | 0.021 | 0.72 | 5.0 | 12 |
| | ±0.8 | ±0.002 | ±0.06 | ±0.1 | |
| | | NaCl 0 mM | | | |
| 2.5 | 0.89 | 0.0033 | 0.90 | 5.09 | 14 |
| | ±0.07 | ±0.0003 | ±0.06 | ±0.04 | |
| 27.5 | 5.1 | 0.017 | 0.85 | 5.0 | 14 |
| | ±0.4 | ±0.001 | ±0.05 | ±0.1 | |
| 37.5 | 8.5 | 0.030 | 0.77 | 5.22 | 17 |
| | ±0.6 | ±0.002 | ±0.05 | ±0.06 | |

* For experiments in NaCl-free solution, the weight of the sucrose in the extracellular space (1) has been subtracted from the dry weight, in order to make values of $J_K$ from these muscles directly comparable to other values in the table.

## RESULTS[1]

Table I presents $K^{42}$ exchange in the steady state as a function of temperature in the presence and absence of NaCl. In the presence of NaCl the steady state is disturbed at low temperatures, due to inhibition of active Na extrusion (1). Accordingly, measurements in the presence of NaCl have not been carried below 17.5°C, at which temperature a steady state with respect to cellular K concentration still prevails. In the absence of NaCl, the cellular K concentration remains at its physiological value even at very low temperatures (1), so that measurement of steady state K exchange can be extended down to 2–3°C.

At 37.5°C in solutions containing NaCl some muscles undergo a net loss of K. Since steady state kinetics are no longer applicable under these conditions, only muscles with K contents $\geq$ 320 mmol/kg dry weight at the end of the experiment were accepted, four muscles being rejected by this criterion.

In Figs. 1 *a* and 1 *b* the dependence of $J_K$ on muscle diameter, $d$, is plotted

[1] Results in the text, Table I, and Fig. 2 are expressed as mean ± standard error. Differences are stated as being significant when $P < 0.01$ using the Student $t$-test.

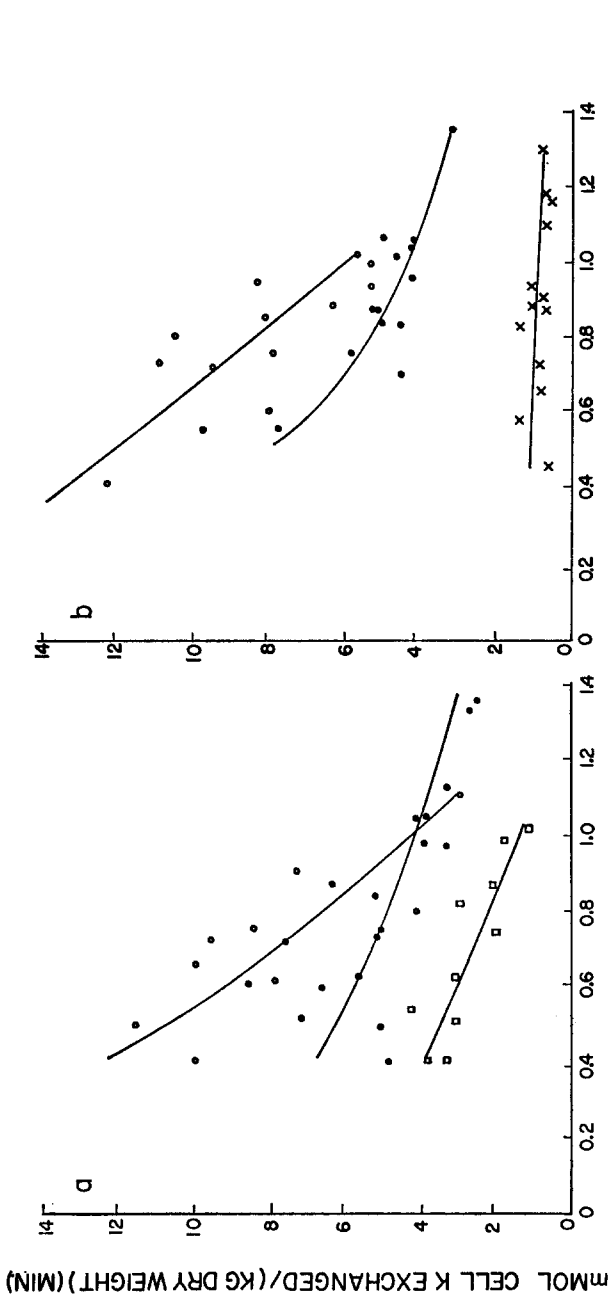


FIGURE 1. Dependence of $J_K$ on muscle diameter in normal Ringer's solution (Fig. 1 *a*) and in NaCl-free solution (Fig. 1 *b*). Reading from the top graph down, curves represent, in order, experiments at 37.5°C (open circles), at 27.5°C (solid circles), at 17.5°C (open squares), and at 2–3° C (×). Curves have been drawn by eye to give best fit.

for the experimental temperatures both in the presence and absence of NaCl. Using an IBM 1620 digital computer, the data were also fitted to a line $y = a + bd$, to a logarithmic relation of the form, $\ln y = a + bd$, and to a polynomial, $y = a_0 + a_1 d + a_2 d^2 + a_3 d^3$. The experiments at 2 to 3°C in NaCl-free solution showed no significant correlation ($R = 0.24$) between $J_K$ and $d$, whatever approximation was used to fit the data. For all other experiments

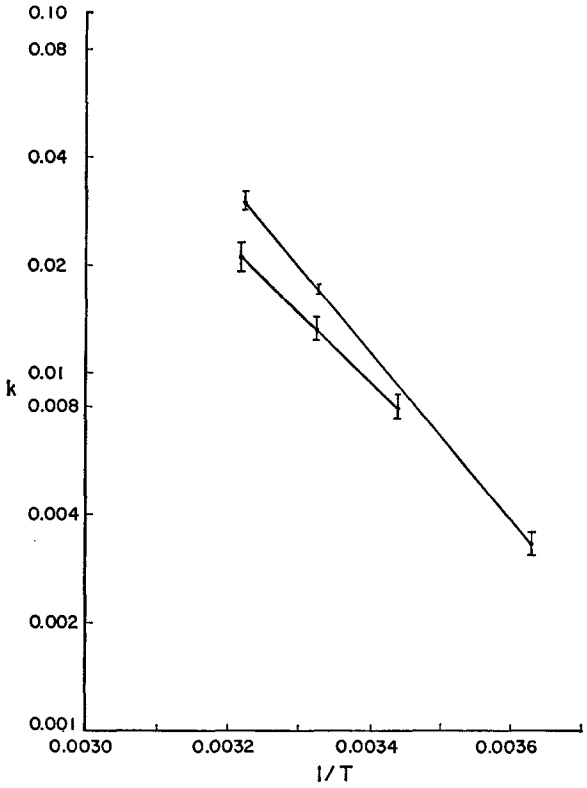


FIGURE 2. Plot of $k$ *vs.* $1/T$. Upper graph, NaCl-free solution, lower graph, normal Ringer's solution.

highly significant correlations ($R = 0.71$ to 0.91) were obtained. The fit to an exponential or polynomial was found to be no better than that to a line. Since none of the three mathematical models successfully predicts the experimental results, curves have been drawn by eye to give the best fit to the experimental points. When muscle diameter is taken into consideration, $J_K$ at 27.5°C as determined by the present method is not statistically different ($P > 0.05$) from the value found in the preceding paper (3).

Fig. 2 is an Arrhenius plot in which the logarithm of $k$, the fraction of cell K exchanged per minute, is shown as a function of the reciprocal of the absolute

temperature. The figure demonstrates that the semilogarithmic plot of the data for NaCl-free sucrose is linear over the range from 37.5 to 2–3°C. The plot of the three points (37.5, 27.5, 17.5°C) in NaCl-containing solution is also linear. From the slopes of the two lines, apparent activation energies (cal/mole) of 8,800 and 10,200 may be calculated for the control and NaCl-free solutions, respectively. The larger exchange constants in NaCl-free Ringer's (Table I) reflect the lower cellular K content in this medium. A smaller cell K content without a change in internal K concentration is brought about by the net outward diffusion of Cl ions as KCl in association with the osmotic equivalent of water, the loss of K with Cl being required to preserve electroneutrality.

It is apparent from Table I that $J_K$ (in NaCl-free solution) at 37.5°C and at 27.5°C is not affected by total replacement of external NaCl by sucrose. $J_K$ in NaCl-free solution at 17.5°C may be computed from the product of $k$ obtained from Fig. 2 and the cell K content (296 ± 5 mmols/kg dry weight) (1). The value of $J_K$ so obtained (for the NaCl-free medium) (in mmols cell K exchanged/(kg dry weight) (minute)) is 2.7, in agreement with the experimental figure of 2.7 at 17.5°C in NaCl-containing solution (Table I). $J_K$ is therefore independent of the presence of external NaCl over the entire temperature range for which a steady state can be maintained.

Page, Goerke, and Storm (1) have shown that $10^{-5}$ M ouabain, which causes a large net loss of cell K in NaCl-containing solution, does not alter the cellular K concentration in NaCl-free sucrose Ringer's. Exploratory flux experiments with the present method in the NaCl-free medium indicated that $J_K$ and $k$ are not altered by this high ouabain concentration. This result is in sharp contrast to the striking reduction in $K^{42}$ influx observed in NaCl-containing media (3).

## DISCUSSION

### *Temperature Dependence of K Exchange*

In the presence of NaCl the $Q_{10}$ for K influx may be calculated from Table I to be 1.6 between 37.5 and 27.5°C, and 1.8 between 27.5 and 17.5°C. Carmeliet (8) gives a $Q_{10}$ of 1.86 for the K influx of sheep heart Purkinje fibers in a medium with 5.4 mM K, calculated from measurements at 4 and 37°C. Taylor (9), who does not state the temperature range studied, reported a $Q_{10}$ of 2.2 for K exchange in isolated, perfused rat hearts. The temperature coefficient of $J_K$ in cat papillary muscle is therefore in satisfactory agreement with that in the other two mammalian heart muscle preparations in which cell K has been shown to exchange at a single rate.

The observed values of the $Q_{10}$ for K exchange are somewhat higher than the figure for diffusion of K in free aqueous solution. The observation that the Arrhenius plot in the absence of NaCl is linear over the range 2.5 to 37.5°C

is consistent with the interpretation that the same single rate-limiting process, presumably permeation of the cell membrane, is being measured at both high and low temperatures. Moreover, the fact that both the magnitude and temperature dependence of the process are effectively unaltered by total removal of NaCl suggests that the process is not coupled to active transport of Na. As pointed out by Danielli (10), the magnitude of the temperature coefficient does not permit differentiation between diffusion and chemical reaction. In this connection, it may be of interest that the activation energy (cal/mole) for diffusion of ions in ion exchangers is of the order 6000 to 10,000 (11), as compared to 4230 for diffusion of K in free aqueous solution (12).

*The Nature of K Exchange in the Steady State*

Page, Goerke, and Storm (1) have shown that incubation in a K-free medium causes a net uptake of Na and a net loss of K by the cells of cat papillary muscle, both processes being reversed by restoring the physiological external K concentration. On the basis of additional experiments in media with varying concentrations of K and ouabain, they suggested that the removal of K ions from a site at the external face of the cell membrane disturbs the physiological steady state by inhibiting active extrusion of Na from the cells. Page, Goerke, and Storm pointed out that the only direct evidence for coupling between movements of Na and K is a K dependence for active transport of Na; the net loss of K when extrusion of Na is inhibited may then be attributed to the diffusion potential set up when NaCl diffuses into the cells.

The results of the present paper indicate that steady state K exchange may readily be studied separately from active transport of Na by replacing all of external NaCl by sucrose. $J_K$ is not affected by this substitution, a finding which would seem to rule out a carrier-mediated forced exchange of K for Na of the type proposed for muscle by Ussing (2, page 59, Fig. 2b). Furthermore, ouabain, which, in the presence of NaCl, inhibits active transport of Na, causes net losses of cellular K, and markedly slows the uptake of $K^{42}$ by papillary muscles (3), does not disturb the steady state or alter $J_K$ in the absence of NaCl. These observations are consistent with the interpretation that ouabain acts primarily on extrusion of Na, the effects on K movements being a consequence of the inhibition of Na transport. According to this view, extrusion of Na is sufficiently active at 17.5°C to maintain the steady state, but becomes impaired at 2–3°C. The present interpretation implies further that the channels by which cellular K exchanges with extracellular K differ from the pathway by which actively transported Na leaves the cells.

The evidence presented in this paper against a forced K-Na exchange leaves the observation that the membrane potential ($V_m$) is less than the K equilibrium potential ($V_K$) (13) as the principal difficulty in the way of the assumption of a passive distribution for K ions. Because of the possibility that this

discrepancy may result from a contribution by the Na ion to $V_m$, it would be of great interest to compare $V_m$ with $V_K$ in NaCl-free sucrose solutions. Measurement of $V_m$ in this medium has not, however, proved technically feasible because the tip potentials of glass microelectrodes are very large in solutions of low ionic strength. The unequivocal demonstration of a passive distribution for K ions by this method must therefore await the development of microelectrodes (perhaps made of glass with different properties) which are not subject to this kind of interference.

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