Cat Heart Muscle *in Vitro*

VI. *Potassium exchange in papillary muscles*

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ABSTRACT The exchange of cell K with K^{42}, J_K, has been measured in cat right ventricular papillary muscle under conditions of a steady state with respect to intracellular K concentration. Within the limits of the measurement, all of cell K exchanged at a single rate. Cells from small cats are smaller and have larger surface/volume ratios than cells from large cats. The larger surface/volume ratio results in larger flux values. J_K increases in an approximately linear manner as the external K concentration is increased twentyfold, from 2.5 to 50 mM, at constant intracellular K concentration. The permeability for K ions, P_K, calculated from the influx and membrane potential, remains very nearly constant over this range of external K concentrations. J_K is not affected by replacement of O_2 by N_2, or by stimulated contractions at 60 per minute, but K influx decreases markedly in 10^{-5} M and 10^{-6} M ouabain.

Previous papers from this laboratory have described the behavior of cellular ion concentrations, cell volume, and resting transmembrane potential difference of cat papillary muscles under various conditions (1, 2). The present report is concerned with the measurement of the exchange of cellular K with extracellular K^{42} in the steady state characterized by a constant cellular K concentration. A kinetic analysis of cellular K exchange, including both the influx and efflux of K^{42}, gives results consistent with a single rate constant for the exchange. The flux of K depends on the diameters of the muscles and cells and on the external K concentration. The effects of electrical stimulation and of anaerobiosis have also been studied.

METHODS

The experiments were designed to measure directly the β-radiation due to K^{42} in a continuously perfused muscle, for the purpose of following the uptake or loss of K^{42} by the muscle as a function of time. Cats weighing 0.8 to 3.0 kg were anesthetized with pentobarbital and their hearts were dissected at room temperature in the cham-

ber described by Page and Solomon (1). Before each experiment, enameled cupron wires (diameter 0.008 cm), threaded into the muscle holder shown in Fig. 1 *a*, were tied to the valvular and mural ends of the papillary muscle with 6–0 surgical silk.

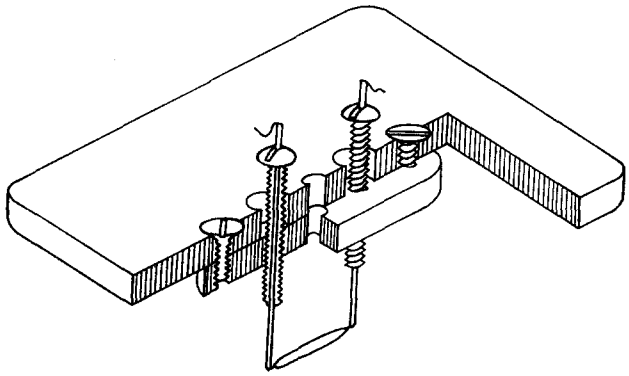

FIGURE 1 *a*. View of muscle holder with part of top plate cut away to show papillary muscle. The ends of the muscle are attached to cupron wires threaded through hollow stainless steel binding posts. The crimped ends of these wires may be seen emerging from the top of the posts. Just below the points of emergence are two screws for adjusting the height of the muscle.

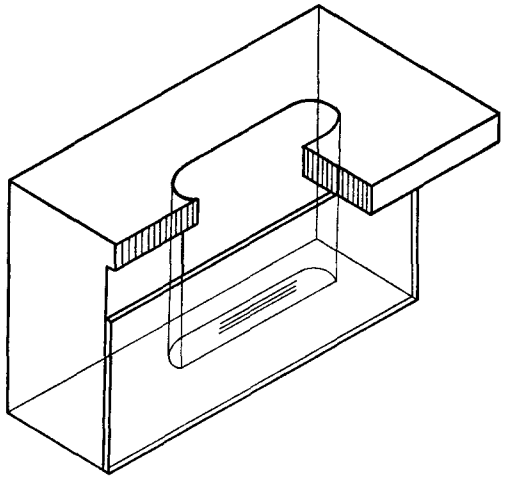

FIGURE 1 *b*. View of positioning chamber without muscle holder. The lower half of the chamber is filled with Ringer's solution. The top plate of the muscle holder rests on the flat upper lucite plate of the chamber. The muscle is suspended in the solution by the binding posts of the holder, and can be positioned by reference to the guidelines etched on the glass window of the chamber. The chamber can be observed by placing it on a tilted microscope stage.

The muscle was cut free of the heart, drawn into position on the holder, and secured in place (stretched to approximately *in situ* length) by crimping the wires at appropriate points. Next, the holder was briefly transferred to a positioning chamber (Fig. 1 *b*) containing oxygenated Ringer's solution. The muscle holder was fitted

with screws which permitted the axis of the muscle to be set at a predetermined distance from the top of the positioning chamber. The adjustment could be made by eye or under microscopic guidance (× 100) on the stage of a microscope. In several trial experiments the critical distance of the muscle from the top of the chamber was found not to vary significantly.

Influx Experiments For K^{42} influx experiments the holder was transferred to an equilibration chamber in which the muscle was perfused at 27.5°C for a period of 2 to 3 hours in a solution of selected K concentration. This preequilibration permitted recovery from the dissection and assured that the net movements of ions and water described by Page and Solomon (1) had approached completion prior to the determination of the steady state K exchange. Subsequently the muscle holder was alternately

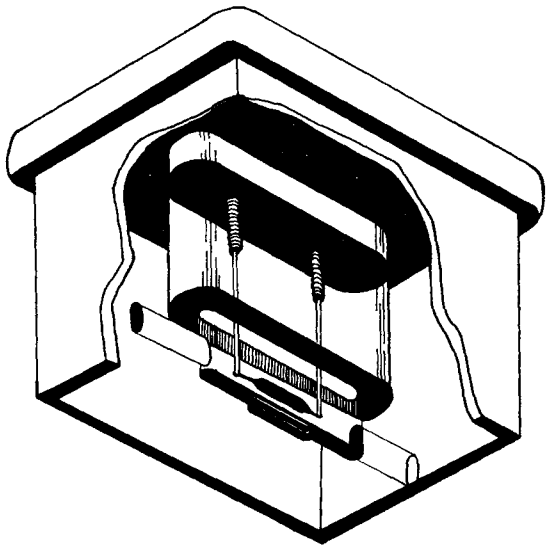

FIGURE 1 *c*. Chamber for monitoring muscle radioactivity. The muscle holder, placed on the flat surface of the chamber top, suspends the muscle in midstream as in the positioning chamber. The geometrical relationship of the muscle to the end window of the Geiger-Mueller tube placed beneath the mylar floor of the chamber is thus maintained. Shielding and counting apparatus are omitted from the drawing in order better to display the muscle.

positioned in an influx bath for labeling with radioactive isotope, and in an assay chamber for monitoring muscle radioactivity. One group of muscles was stimulated at a rate of 60 per minute during the entire period in the influx chamber, using a Grass model S4E stimulator and stainless steel electrodes placed in the perfusing solution adjacent to the muscle. An attempt was made to stretch contracting muscles to approximately their *in situ* length, and the contractions were observed by eye; contractile tension was not recorded.

Efflux Experiments For K^{42} efflux experiments the muscle was transferred directly from the positioning chamber to the influx bath. After a 5 to 6 hour period of equilibration with radioactive solutions (sufficient for complete exchange of muscle

K), the holder was placed in the efflux (assay) chamber where the tissue radioactivity was monitored continuously for up to 2 hours.

After completion of influx or efflux measurements, the muscle was gently blotted and a known length cut from its midportion with two razor blades mounted a fixed distance apart in a small block. The tissue was then dried under reduced pressure with an infrared lamp, weighed on a microbalance (Cahn electrobalance, model M-10), and the fraction of muscle K exchanged with K^{42} determined as described below.

Perfusion Apparatus The perfusion systems were designed for rapid flow rates in order to reduce to a minimum the unstirred surface layers of fluid which might have slowed the exchange between the muscle and the bathing medium. For this reason, the perfusion chamber used for measurement of K^{42} in the muscle was constructed with a fluid path 0.22 cm wide by 0.5 cm deep and was perfused at a volume flow of 150 ml per minute, yielding a linear flow rate in excess of 22 cm per second. Fig. 1 *c* shows this first perfusion system, a lucite efflux chamber which served primarily for monitoring the β-radiation in the muscle, but was sometimes also used for preequilibrating muscles with non-radioactive solutions. The floor of this chamber, formed by a mylar membrane, was the end window of a Geiger-Mueller counter. The total radioactivity of the solution within the assay chamber was always less than 1 per cent of that in the muscle.

A second perfusion system having a volume of 16 ml was used during the influx of K^{42}. In this chamber muscles were allowed to accumulate K^{42} prior to transfer to the first chamber for measurement of the amount of K^{42} taken up. Fluid was circulated through this second perfusion system by means of a large peristaltic action pump (American Instrument Co., model 5-8952). The volume of this influx system was effectively infinite relative to the muscle volume. A modification of this arrangement having a total volume of 500 ml served as an auxiliary chamber for preequilibrating papillary muscles in non-radioactive media.

Measurement of the β-Radiation in Papillary Muscles

The apparatus for monitoring papillary muscle radioactivity was similar to that used by Keynes (3) in frog toe muscles and by Carmeliet (4) in sheep Purkinje fibers. With the holder placed in the efflux chamber, β-radiation from the muscle was assayed by a Geiger-Mueller tube situated below the mylar window. The tube (Anton Electronics No. 224), selected for its low background, had a diameter of only 0.9 cm. It was shielded by a sliding brass plate 0.238 cm in thickness. An elliptical aperture, 0.121×0.198 cm, served to exclude extraneous radiation and permitted only β-radiation from the intact midportion of the muscle to be counted. By mounting the entire assembly inside a lead box, the background was lowered further to 1 to 2 counts per minute. After removal of a radioactive muscle following experiments with K^{42} the background was only 6 counts per minute. The output of the Geiger-Mueller tube was connected to a scaler (Nuclear Chicago, model 161 A), the register signal of which was fed into a ratemeter (Berkeley, model 1600) and thence to a strip recorder (varian, model G-11 A). Corrections were applied for the dead time of the tube, ratemeter calibration, background, and isotopic decay. The decay correction,

timed at the midpoint of each 4 to 6 minute interval in the assay chamber, was applied to the entire interval. Ratemeter calibration curves were obtained by comparing the readings of the scaler and ratemeter for a given input from a signal generator.

The γ-radiations of the loading solution and of the dried muscle segment at the end of each experiment were compared on the following day using a well-type scintillation counter, this information being required for determining muscle specific radioactivity.

Solutions

The experimental solutions were the Cl-Ringer media described by Page and Solomon (1). The composition of the physiological control solution (mM) was 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 glucose 5.5. The K concentration of this solution was varied from 1 mM to 50 mM by a mole for mole substitution of KCl for NaCl or of NaCl for KCl. Except for the brief time in the positioning chamber, these solutions were continuously equilibrated with 95 per cent O_2, 5 per cent CO_2 or 95 per cent N_2, 5 per cent CO_2 as required to maintain the pH between 7.2 and 7.4. For a given experiment solutions differed only in the presence or absence of radioactive tracers, and were of identical chemical composition in the baths used for preequilibration, influx, and efflux. The various baths were maintained at the desired temperature of 26–28°C by water circulated through condenser jackets from a thermostated reservoir.

K^{42} was received as the chloride from Iso/Serve, Inc., Cambridge, Massachusetts. The carrier concentration of K, determined by flame photometry after each experiment on a diluted aliquot of the bathing solution, was frequently found to differ appreciably from the value stated by the supplier. In tabulating the experimental results, the external K concentration, $[K]_o$, is accordingly given as the mean value $\pm$ standard error.

Chemical Analysis

Muscles were extracted with 0.1 N HNO_3, and distilled water solutions of the dried extract were prepared as previously described (1). The K concentrations of these extracts and of appropriate dilutions of the bathing medium were determined with a flame photometer.

Histological Methods

Seven papillary muscles, from five cats varying in weight from 0.40 to 3.6 kg, were tied with silk thread to special holders made by bending glass capillaries (A. S. Aloe Co. V48302) into the shape of the metal holders used for incubation by Page (5). The muscles were carefully stretched to approximate their *in situ* resting length. Muscle diameters were measured in Ringer's solution as described in the accompanying paper (6), and the glass holders were then plunged into the muscle fixative (formalin buffered to pH 7.4). Paraffin-embedded transverse sections, stained with hematoxylin and eosin, were prepared from these muscles through the courtesy of Dr. J. M. Corson, Department of Pathology, Peter Bent Brigham Hospital. Areas of transversely cut myocardial cells were examined microscopically under oil immersion ($\times$ 1000). For each muscle the average number of transversely sectioned nuclei per microscopic field was determined by counting the number of such nuclei in nine fields.

Calculations

DERIVATION OF THE K EXCHANGE RATE

(*a*) *Derivation from Influx Measurements* To calculate the steady state exchange rate of cellular K, J_K, it is necessary to determine the fraction of muscle K exchanged with K^{42} as a function of time. The two experimentally obtained quantities are the rate constant for the exchange, k (in units of (time)$^{-1}$) and the cellular K content[1] per kg dry weight, K_m. The product kK_m is equal to J_K, which, in the steady state, is equal to both influx and efflux [in mmols cell K/(kg dry weight) (min.)]. f, the fraction of muscle K which had equilibrated with K^{42} from the influx bath, was determined at the end of the influx experiment from the ratio of specific radioactivities in the muscle and loading solution according to the relation

$$f = (K_m^*/K_m)/(K_o^*/[K]_o V_o) \quad (1)$$

in which

K_m^* = the counts per minute (CPM) per kg dry weight in the segment of muscle counted by the Geiger-Mueller tube. (K_m was subsequently measured chemically in the same segment on which K_m^* had been previously determined.)

K_o^* = the CPM in an aliquot of the influx bath (both K_m^* and K_o^* are corrected for background and radioactive decay). Since the volume of the bath was very large relative to that of the muscle, K_o^* was effectively constant during the experiment.

$[K]_o$ = the K concentration of the influx bath, and

V_o = the volume of the aliquot of the influx bath.

Records of the output of the ratemeter during an influx, obtained after each interval of equilibration with K^{42}, consisted of multiple curves corresponding to 4 to 6 minute periods of washout of part of the radioisotope in the muscle into non-radioactive solution in the assay chamber. The values of K_m^* were derived from records of the ratemeter output as follows: Each washout was resolved into two exponential rates, and the exponential characterizing the slower rate was graphically extrapolated to the time at which the muscle was changed from the radioactive loading solution to the inactive monitoring bath. The intercept on the ordinate was taken as the cellular radioactivity at the beginning of the counting interval. $K_{m\infty}^*$, the value of K_m^* when all of cell K has exchanged, was then derived by dividing the CPM in the muscle segment at the end of the experiment by f. The rate constant for cellular K exchange, k, was taken as the slope of the linear plot of $\ln(1 - (K_m^*/K_{m\infty}^*))$ against time.

Since the procedure of radioactive measurement was necessarily associated with an efflux of K^{42} into the non-radioactive solution perfusing the assay chamber, a part of

[1] Because extracellular K comprises only a very small fraction of muscle K, cell K was taken as equivalent to K_m, the K content of the muscle segment, and the counts due to β-radiation in the cells as equivalent to K_m^*, the corrected counts per minute in the muscle segment. After a sufficiently long period of time, the error in K_m and K_m^* introduced by this approximation is small. Taking the extracellular space as the inulin space (1), extracellular K is only 1.4 per cent of total K for an external K concentration ($[K]_o$) of 5.3 mM, and becomes entirely negligible when $[K]_o$ is 2.5 or 1 mM. At $[K]_o$ = 50 mM, the highest K concentration used, extracellular K is only 7 per cent of total K.

the next loading interval was taken up in recovering these counts. The time used in regaining K^{42} lost during the preceding measurement was therefore calculated by a graphical method (Fig. 2) and subtracted from the duration of the loading interval.

(*b*) *Derivation from Efflux Measurements* The rate constant for K exchange in efflux experiments was obtained from the slope of the continuous plot of $\ln(K^*_m/K^*_{mo})$ against time in which K^*_{mo} is CPM/kg dry weight at time zero. By the end of the efflux,

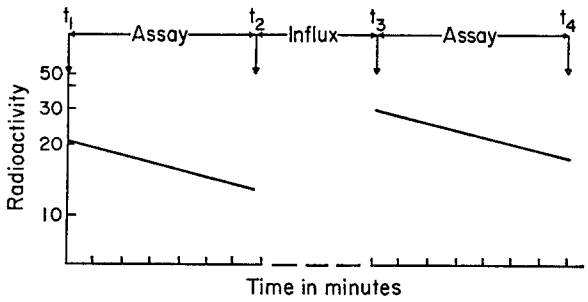

FIGURE 2. Illustration of rationale for correcting time base. Abscissa time in minutes; ordinate, muscle radioactivity relative to a value of 100 at specific activity equilibrium. Except during the very early portion of the influx measurement, the duration of the variable influx (loading) interval (broken time axis) was long relative to the period used for assay. The ordinate has been corrected for background, for radioactive decay, and for the rapidly declining (extracellular) component. At t_1 the muscle was transferred from the influx bath labeled with K^{42} to the assay chamber in which it was perfused with non-radioactive solution until time t_2. It was then returned to the influx bath for further uptake of K^{42}. The assay was repeated during the interval t_3–t_4. The radioactivity at t_3 is lower than it would have been if K^{42} had not been lost during the assay period t_1–t_2. The time interval of interest is the number of minutes required to raise the radioactivity of the muscle from the value at t_1 to that at t_3. From the linear plot of $\ln(1 - f)$ against time the duration (minutes) required to take up the K^{42} lost during interval t_1–t_2 is calculated, and this value is subtracted from the loading interval t_2–t_3. The extracellular compartment was assumed to reequilibrate completely during each loading (labeling) interval because of the rapid extracellular diffusion rate (8) and the duration of the loading intervals, which were long relative to the intervals used for assay. An analogous correction of the time base for the extracellular component of the washout was therefore unnecessary.

a large fraction of muscle K^{42} had been washed out, and f was consequently small. In order to derive the value of f applicable before the beginning of the efflux (at $t = 0$), the value of f at the end of the efflux was multiplied by R_0/R_2 in which R_0 and R_2 are, respectively, the corrected CPM on the ratemeter at the beginning and end of the efflux. The value of fR_0/R_2 was found without exception to fall between 0.93 and 1.02, as would be expected if the efflux had begun with the specific radioactivity of muscle K equal to that of the bathing solution.

Muscle diameters were calculated from the length and weight of the muscle segment, using the specific gravity of mammalian muscle (7) and assuming cylindrical

geometry. In selected experiments, diameters were measured directly by the method described in (6).

RESULTS[2]

Control Experiments

Derivation of the K exchange flux by the above methods assumes that for each assay period all of cell K exchanges with a single rate constant, and that the cellular K concentration is constant during the entire flux measurement. In

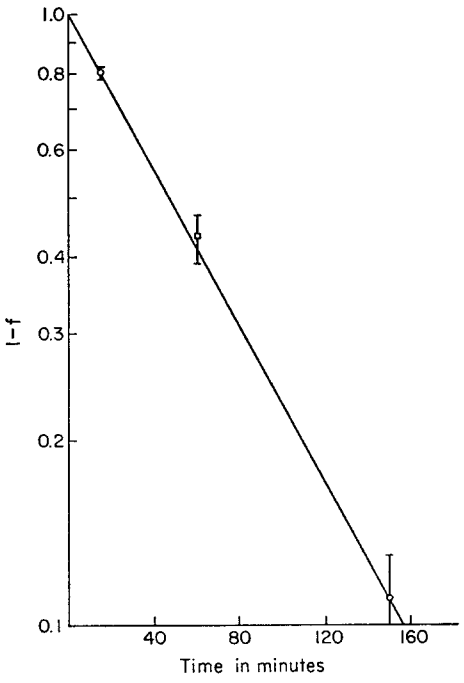

FIGURE 3. Exchange of cellular K with K^{42}. The points at 15 and 150 minutes are the results of influx determinations on two series of fifteen and fourteen muscles, respectively, measured as described in the accompanying paper (6). The point at 60 minutes is the result of sixteen efflux experiments (see text).

Fig. 3, ln $(1 - f)$, the logarithm of the fraction of cell K remaining unequilibrated, is plotted against time. Two points have been obtained by a method described in the accompanying paper (6), the first point early in the influx (15 minutes of equilibration, 20 per cent exchange), the second point near specific activity equilibrium (150 minutes of equilibration, 80 per cent exchange). As would be expected if all of cell K exchanges with a single rate constant, the two points lie on a line which extrapolates to $(1 - f) = 1$ when $t = 0$. In Fig. 3, the point at $t = 60$ minutes is the mean of sixteen efflux experiments in which muscles were preequilibrated for 5 hours in radioactive loading solution to assure equal specific activities of K in muscles and bath. The mus-

[2] Results in tables, figures, and text are expressed as mean ± standard error. Significance of differences is analyzed using the Student *t*-test.

cles were then immersed for 1 hour in a large volume of non-radioactive solution, blotted, and assayed for K^{42} in a well-type scintillation counter and for total K content as described above. The value of ln $(1 - f)$ at 60 minutes from this efflux measurement clearly yields the same exchange constant as the influx measurement. On the basis of the results shown in Fig. 3, cell K will be treated as exchanging uniformly, with the understanding that a slower ex-

TABLE I*

K EXCHANGE UNDER VARIOUS CONDITIONS (26–28°C)

Experiment	J_K	k	Muscle diameter	$[K]_o$
	mmols cell K exchanged / (kg dry weight) (min.)	*(min.)*$^{-1}$	*mm*	*mM*
Control influx (7)	3.5	0.0088	1.07	5.4
	±0.3	±0.0008	±0.06	±0.2
Control efflux (6)	3.9	0.010	0.96	5.2
	±0.5	±0.001	±0.03	±0.1
Influx stimulated at 1 per sec. (6)	3.7	0.0089	1.09	5.5
	±0.1	±0.0004	±0.04	±0.1
Influx (nitrogen) (5)	3.7	0.010	0.99	6.5
	±0.5	±0.001	±0.04	±0.4

* Figures in parentheses give number of experiments.

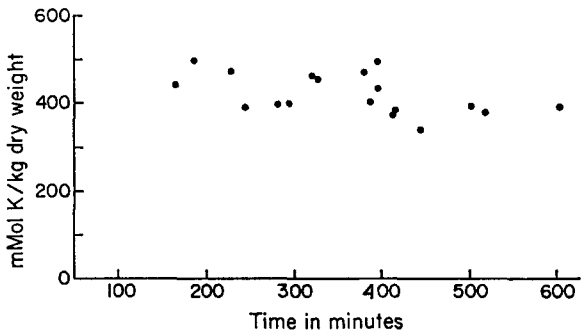

FIGURE 4. Muscle K content as a function of the duration of incubation in the perfusion system.

change of a small fraction of cell K cannot be ruled out. Even if such a compartment exists, the values of k (Tables I and III) would still describe the exchange rate for the bulk of cellular K.

Fig. 4 shows the muscle K content, K_m, for eighteen muscles in the control solution, plotted as a function of the duration of incubation. It is apparent from the figure that the steady state assumption with respect to cell K was approximated for nearly 10 hours in this group of muscles. In Table I the control values for J_K, measured both as influx and efflux, are seen to be the

same ($P > 0.5$). The influxes were measured during the early hours of equilibration, while efflux measurements were not begun until 5 to 6 hours later. The identity of the values of J_K obtained by the two methods thus confirms that the flux was approximately time-invariant. This conclusion also receives support from the efflux experiments plotted as the 60 minute point of Fig. 3, which yields similar values of J_K.

Dependence of J_K and k on Muscle Diameter

At an early stage of these measurements it was observed that J_K and k varied inversely as the muscle diameter. This phenomenon was found to persist under

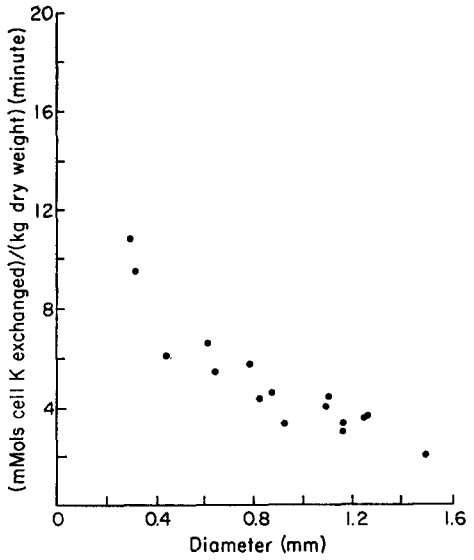

FIGURE 5. Plot of J_K against muscle diameter for a group of muscle preincubated for 5 hours in physiological Ringer's solution. J_K and diameter were measured as described in the accompanying paper (6). Temperature 27.5°, $[K]_o$ = 5.2±0.1 mM.

all the experimental conditions used in this and the accompanying paper (6). A typical example is shown in Fig. 5, in which J_K is plotted against the muscle diameter (d) for sixteen muscles preincubated for 5 hours in control solution. The dependence of J_K on d is described further in the accompanying paper and summarized graphically ((6), Fig. 1).

Because the extracellular diffusion path for K^{42} is longest in those muscles having the largest diameters, delayed extracellular diffusion was entertained as a possible cause of the inverse relation between the muscle diameter and J_K or k. The analysis of Keynes (3) indicates that for cylindrical tissues with diameters as small as those of the muscles used in the present experiments extracellular diffusion delays should be too small to be detected by our assay system. Moreover, this explanation could readily be excluded by the following two considerations: (*a*) Experiments invariably showed the rapid phase of K^{42} efflux into non-radioactive solutions to be brief, consistent with an extracellular diffusion rate for K equal to approximately half that in free aqueous solu-

tion (8). Using the diffusion equation for cylindrical muscles given by Hill (9), the time constant for extracellular equilibration could be shown to be small relative to the influx and efflux times used in the present study. (*b*) The dependence on muscle diameter was also observable in efflux experiments long after the disappearance of the extracellular component of the washout and under conditions in which extracellular K^{42} accounted for less than 1 per cent of muscle radioactivity.

An alternative explanation for the inverse dependence on muscle diameter was suggested by the fact that muscles with larger diameters were, in general, obtained from larger cats. If the cells in muscles with larger diameters are larger, they should have smaller surface to volume ratios, yielding smaller values of J_K and k. Table II summarizes the results of cell counts on transverse sections of muscles with varying diameters obtained from cats of different

TABLE II

DEPENDENCE OF CELL SIZE ON WEIGHT OF CAT AND MUSCLE DIAMETER

No. of cell nuclei per microscopic field (× 1000)	Weight of cat	Diameter
	kg	*mm*
40±1	0.400	0.98
32±1	1.37	1.06
35±1		0.87
25±1	1.70	1.18
23±1		2.00
19±1	2.20	1.48
13±1	3.60	>2.00

sizes. The cell count was approximated from the number of nuclei cut in cross-section, as already described. This approximation assumes that shrinkage and distortion in the preparation of the stained sections affect large and small cells to the same extent and that the correspondence between number of cells and number of nuclei is approximately the same for each section. The table clearly shows a progressive decrease in the number of cells per microscopic field as the animals' weights increase. It is also of interest that there appear to be more cells in the smaller of a pair of papillary muscles from the same right ventricle, although the data do not suffice to establish this point statistically. On the basis of these observations it was concluded that muscles with larger diameters were characterized by a smaller cell surface to volume ratio, and that this would result in smaller values for J_K.

Effect of Stimulation

At 27–28°C stimulated papillary muscles remain in a steady state with respect to cellular K concentration (1). If a transient net efflux of K occurs in associ-

ation with the action potential, the attendant loss of cell K must therefore be reversed by a net uptake of K during the intervals between action potentials. Provided these processes involve a sufficiently large fraction of cell K, a transient net K efflux followed by a transient net K influx should be observable by an increase in J_K and k.

Table I shows no significant change in J_K when papillary muscles were stimulated at a frequency of 60 per minute. This suggests that no net K flux occurs with stimulation or that any net fluxes of K are too small or localized to be detected by the experimental arrangement used. Our findings are in agreement with those of Taylor (10) in perfused rat hearts, with those of Langer and Brady (11) in perfused dog papillary muscle, and with unpublished results of Brady and Woodbury (12) on K efflux in frog hearts. The present results are at variance with the interpretations of Lorber *et al.* (13) and of Danielson, Öbrink, and Sjöstrand (14) based on *in vitro* measurements in frog hearts. The latter two groups found a phasic cellular efflux of K with each action potential, the quantity measured being K^{42} diffusing out of the extracellular space. Such an efflux might arise without an increased cellular K exchange, if the extracellular compartment, which is unstirred in quiescent muscle, is mechanically agitated by cardiac contractions.

K Exchange in Absence of Oxygen

It is apparent from Table I that K influx in non-contracting muscle is not affected by substitution of 95 per cent N_2-5 per cent CO_2 for the usual 95 per cent O_2-5 percent CO_2 gas mixture. It has previously been suggested by us (15) that exhaustion of cellular reserves of high energy compounds appears to be a prerequisite for the disturbance of the steady state and consequent net losses of K in absence of oxygen. Failure to exhaust these stores is the probable explanation for the present experimental observations. By contrast, exploratory experiments in oxygenated solution containing 10^{-5} M or 10^{-6} M ouabain, which disturbs the steady state by inhibiting extrusion of Na (15), invariably revealed a diminution of the K^{42} uptake. Since J_K and k have been defined for steady state conditions, their values cannot be calculated during the action of ouabain which characteristically disturbs the steady state. A quantitative comparison between K influx in the steady state and under the ouabain-induced non-steady state conditions is therefore not possible. Such a comparison would require a much more detailed description of the time dependence of the membrane potential and cellular ion concentrations during ouabain inhibition.

K Exchange as a Function of the External K Concentration

It has previously been shown that the intracellular K concentration, $[K]_i$, remains at its physiological value when the external K concentration, $[K]_o$, is varied from 2.5 to 50 mM by a mole for mole substitution of KCl for NaCl,

and sufficient time is allowed for a new steady state to become established. We have therefore determined J_K after a 2-hour period of preequilibration in solutions having a $[K]_o$ varying from 2.5 to 50 mM. It thus became possible to study J_K as a function of $[K]_o$ at constant $[K]_i$, under the same conditions already used for measurements of the resting potential (V_m) (2) and cell volume (1).

In Table III the results of such measurements are assembled in order of increasing $[K]_o$. Since the mean muscle diameters at values of $[K]_o$ of 5.4 mM, 25.5 mM, and 50.2 mM are statistically equal, the mean values of J_K (for these values of $[K]_o$) have been plotted *vs.* $[K]_o$ in Fig. 6. At $[K]_o$ = 2.43, J_K has

TABLE III

DEPENDENCE OF K EXCHANGE ON $[K]_o$ AT 26–28°C*

$[K]_o$	J_K	k	$P_K/P_{(K=5.4)}$	Diameter	No. of experiments
mM	*mmols cell K exchanged / (kg dry weight) (min.)*	$(min.)^{-1}$		*mm*	
2.43 ±0.07	2.7 ±0.2	0.0068 ±0.0009	1.2‡	0.89 ±0.06	7
5.4 ±0.02	3.5 ±0.3	0.0088 ±0.0008	1.0	1.07 ±0.06	7
25.5 ±0.2	12.4 ±1.1	0.022 ±0.005	1.2	1.12 ±0.08	8
50.2 ±0.9	23 ±2	0.044 ±0.004	1.5	1.08 ±0.08	10

* Values of V_m (in mv) used in the calculation of P_K were 88.0, 80.0, 43.3, and 24.9, respectively, corresponding to values of $[K]_o$ (in mM) of 2.43, 5.4, 25.5, and 50.2 (2).
‡ Calculated for J_K = 2.0 mmols cell K/(kg dry weight) (min.), corresponding to a diameter of 1.09 mm.

been calculated for a diameter (d) of 1.09 mm, using the linear regression equation of $J_K = 5.43 - 3.14d$ obtained from the experimental measurements of J_K and d at $[K]_o$ = 2.43. The point thus obtained is seen to fall on the same line as the flux values at higher $[K]_o$. J_K therefore increases linearly over a twentyfold range of $[K]_o$.

The increase in J_K with $[K]_o$ in the present study may be compared with the measurements of Carmeliet (4) in isolated Purkinje fibers from the sheep heart. In Carmeliet's study, an increase in $[K]_o$ from 2.7 to 50 mM at 37°C resulted in a 7.4-fold increase in J_K, measured as an influx. A corresponding change of $[K]_o$ at 27.5°C from 2.43 to 50.2 mM in cat papillary muscle leads to an 8.5-fold increase in K exchange (Table III).

The constant field hypothesis of Goldman (16) and of Hodgkin and Katz (17) predicts that the tracer influx of K in the steady state, J_K', expressed in units of (mmols cell K exchanged)/ (cm^2 cell surface area) (min.), should be

related to V_m and $[K]_o$ by

$$J'_K = (P_K F V_m / RT)([K]_o / (1 - e^{-V_m F/RT})) \quad (2)$$

V_m being taken as $V_{out} - V_{cell}$. Equation 2 was derived for ion movements by diffusion under a gradient of electrochemical potential; it does not take into account effects on K influx of cell volume changes, active transport, exchange

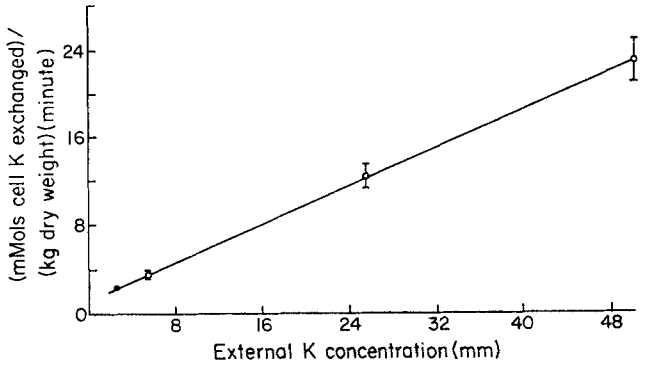

FIGURE 6. Plot of J_K as a function of the external K concentration. The line has been drawn by eye through the mean values of J_K corresponding to the mean values of $[K]_o$ at 2.5, 5.4, 25.5, and 50.2 mM. It does not significantly differ from the line defined by the regression equation $J_K = 0.98 + 0.44\ [K]_o$ (coefficient of correlation + 0.92) derived from the plot of J_K for each experiment at the experimental value of $[K]_o$ for that experiment.

diffusion, or single file diffusion. J'_K is related to J_K determined in the present experiments by

$$aJ'_K = J_K \quad (3)$$

in which a is the cell surface area per kg dry weight (cm^2/kg). Using the values of V_m determined by Page (2), equations 2 and 3 were solved for P_K. The results are assembled in Table III, P_K being expressed in relative (dimensionless) units with respect to a value of unity in physiological solution ($[K]_o$ = 5.4 mM). Assuming that a is on the average the same for all our muscles, it is apparent that P_K is nearly constant over the tenfold range of $[K]_o$ from 2.5 to 25 mM and increases slightly at 50 mM. These findings are in qualitative agreement with those of Carmeliet (4) in sheep Purkinje fibers.

DISCUSSION

Exchangeability of Cell K

Before comparing absolute values of J_K in papillary muscles with the results of other studies, it is necessary to discuss differences in the exchangeability of cell K reported in other investigations of K exchange in *in vitro* preparations of heart muscle. It was found in the present study that at least 90 per cent of cell

K exchanges with a single rate constant. This finding agrees with the observations of Carmeliet (4) on single Purkinje fibers from sheep hearts, of Taylor (10) in perfused rat hearts, and of Burrows and Lamb (18) on chick embryo heart muscle cells in tissue culture. It is also consistent with the results of Hodgkin and Horowicz (19) in single fiber preparations of frog skeletal muscle. On the other hand, at least two rates of exchange for cellular K have been reported by Schreiber and coworkers in frog (20) and guinea pig (21) hearts, by Humphrey and Johnson (22) in rabbit hearts, by Haas and Glitsch (23) in frog hearts, and by Weatherall (24) in rabbit auricles. The *in vitro* preparations used by these investigators were considerably larger than the papillary muscles used by us or than the unicellular preparations studied by Carmeliet and by Burrows and Lamb. The difference may therefore reside in part in the greater difficulty of separating the extracellular component of the K^{42} washout from the cellular component in these larger and more complex preparations, or of maintaining the cellular K concentration in a steady state. It must be emphasized that the experiments of the present paper do not exclude the existence of cellular subcompartments for K, provided these compartments exchange very rapidly relative to the exchange of cellular with extracellular K across the cell membrane.

The Absolute Value of the K flux

It is instructive to compare the rate of K exchange in cat papillary muscles with that of other mammalian heart muscle preparations in which cell K exchanges at a uniform rate. In a medium with an external K concentration of 5.4 mM, the exchange constant of a single sheep ventricular Purkinje fiber at 37°C was 0.008/min. (Carmeliet (4), Fig. 32), as compared to 0.009 to 0.010/min. at 27–28°C found in the present study, and to the figure of 0.021 at 37.5°C in the accompanying paper (6). The K in myocardial cells from cat right ventricle thus exchanges much more rapidly than that of Purkinje cells. The discrepancy may be accounted for in part by the much larger surface to volume ratio of cat papillary muscle cells. The cell diameter of cat papillary muscle cells is reported by Draper and Mya-Tu (25) as 7 to 14 μ. A difference in cell diameter cannot, however, be adduced as an explanation for the very much larger values of k and J_K observed by Taylor (10). Although the cellular diameter of 8 to 12 μ for rat ventricular cells is comparable to that of cat papillary muscle (26), the exchange constant and flux rate at 27°C reported by Taylor are about five times as large as those of the present study. It is possible that the discrepancy may represent a true species difference between rat and cat heart muscle.

The cell surface of myocardial cells has recently been shown to be very complex (27). It therefore seemed premature to refer flux values for papillary muscles to an arbitrarily defined cell surface area, and the results have accordingly been expressed as flux per kg dry weight. When a reliable figure for

cell surface area becomes available, the present results should be readily convertible to this reference unit.

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BIBLIOGRAPHY

1. PAGE, E., and SOLOMON, A. K., *J. Gen. Physiol.*, 1960, **44**, 327.
2. PAGE, E., *J. Gen. Physiol.*, 1962, **46**, 189.
3. KEYNES, R. D., *Proc. Roy. Soc. London, Series B*, 1954, **142**, 359.
4. CARMELIET, E. E., Chloride and Potassium Permeability in Cardiac Purkinje Fibers, Brussels, Editions Arscia S. A., 1961.
5. PAGE, E., *J. Gen. Physiol.*, 1962, **46**, 201.
6. PAGE, E., *J. Gen. Physiol.*, 1965, **48**, 949.
7. CREESE, R., *Proc. Roy. Soc. London, Series B*, 1954, **142**, 497.
8. PAGE, E., and BERNSTEIN, R. S., *J. Gen. Physiol.*, 1964, **47**, 1129.
9. HILL, A. V., *Proc. Roy. Soc. London, Series B*, 1928, **104**, 39.
10. TAYLOR, I. M., *Fed. Proc.*, 1962, **21**, 144.
11. LANGER, G. A., and BRADY, A. J., *Fed. Proc.*, 1963, **22**, 287.
12. BRADY, A. J., and WOODBURY, J. W., *J. Physiol.*, 1960, **154**, 385.
13. LORBER, V., WALKER, J. L., GREENE, E. A., MINARIK, M. H., and PAK, M. J., *Am. J. Physiol.*, 1962, **203**, 253.
14. DANIELSON, B., ÖBRINK, K. J., and SJÖSTRAND, M., *Acta Soc. Med. Upsalien.*, 1962, **67**, 179.
15. PAGE, E., GOERKE, R. J., and STORM, S. R., *J. Gen. Physiol.*, 1964, **47**, 531.
16. GOLDMAN, D. E., *J. Gen. Physiol.*, 1943, **27**, 37.
17. HODGKIN, A. L., and KATZ, B., *J. Physiol.*, 1949, **108**, 37.
18. BURROWS, R., and LAMB, J. F., *J. Physiol.*, 1962, **162**, 510.
19. HODGKIN, A. L., and HOROWICZ, P., *J. Physiol.*, 1959, **145**, 405.
20. SCHREIBER, S. S., *Am. J. Physiol.*, 1956, **185**, 337.
21. SCHREIBER, S. S., ORATZ, M., and ROTHSCHILD, M. A., *Am. J. Physiol.*, 1961, **200**, 1055.
22. HUMPHREY, E. W., and JOHNSON, J. A., *Am. J. Physiol.*, 1960, **198**, 1217.
23. HAAS, H. G., and GLITSCH, H. G., *Arch. ges. Physiol.*, 1962, **275**, 358.
24. WEATHERALL, M., *Proc. Roy. Soc. London, Series B*, 1962, **156**, 57.
25. DRAPER, M. H., and MYA-TU, M., *Quart. J. Exp. Physiol.*, 1959, **44**, 91.
26. TRUEX, R. D., *in* The Specialized Tissues of the Heart (A. P. de Carvalho, W. C. de Mello, and B. F. Hoffman, editors), New York, Elsevier Publishing Co., 1961, **22**.
27. NELSON, D. A., and BENSON, E. S., *J. Cell. Biol.*, 1963, **16**, 297.