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Compartmental Analysis of Potassium Efflux from Growth-Oriented Heart Cells

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Summary. Radioisotopic flux studies were initiated with a new preparation of growth-oriented heart cells to determine the contribution of heterogeneous cell types and the limitations of extracellular diffusion in quantitating the passive movement of potassium ions. The efflux of potassium-42 from contractile preparations, which contain two populations of cells, cardiac muscle and fibroblastlike, could be resolved into two components similar to that described for naturally occurring preparations of cardiac muscle. Compartmental analysis of the efflux data, using analog and digital computational methods, resolved the tracer kinetics into a slow compartment ($k=0.015$ min^{-1}) associated with fibroblastlike cells and a fast compartment ($k=0.067$ min^{-1}) associated with the cardiac muscle cells. The rate constants derived from compartmental analysis were independent of tracer equilibration and preparation dry weight. Analytical measurements of the preparations provided a quantitative basis for determining the transmembrane potassium fluxes from the tracer kinetics. Cardiac muscle cells stimulated at a rate of 150 min^{-1} in the presence of 5.4 mM external potassium were found to have a potassium efflux of 15.7 pmoles cm^{-2}sec^{-1} whereas the value obtained for the fibroblastlike cells was 1.88 pmoles cm^{-2}sec^{-1}. Diffusional limitations of ^{42}K efflux were analyzed for several important variables which can affect isotopic reflux, namely, transmembrane flux, cell volume-to-surface area and cell packing fraction.

A direct measure and quantitative description of the movement of ions across the cell membrane can be obtained from radioisotopic flux studies provided that isotopic diffusion into and out of the preparation is limited solely by the cell membrane. In multicellular preparations, e.g., cardiac muscle, diffusional limitations resulting from the complexity of the tissue morphology and the heterogeneity of the cell types in a given preparation are factors which can limit the determination of true transmembrane fluxes. As a consequence, it is important to question whether the rate constants measured in multicellular preparations of cardiac muscle accurately represent the true exchange rate of radiotracers across the cardiac cell membrane.

In recent years, a number of investigators have studied the kinetics of radioactive potassium (^{42}K) exchange across a variety of adult cardiac tissue [18, 23, 24, 26]. In general, the efflux of ^{42}K as a function of time from the preparations in these experiments was not describable by a single exponential. Given the morphologic complexity of the preparations, the reported differences in potassium efflux kinetics could perhaps have been attributed to the difficulties in accurately separating the contribution of the various cellular and extracellular compartments. Similar problems were encountered in studies with embryonic heart muscle [4, 21] and as a result, the analyses were only concerned with the values obtained from the slowly exchanging component of the ^{42}K efflux curves presumed to be that of tracer exchange from the functional myocardial cells. Interestingly, single exponential rate constants, comparable in magnitude to those for the slow compartment of the embryonic chick heart [4], were obtained from a secondary cell culture of quiescent, nonstriated cells derived from embryonic hearts [1]. More recently, Cheneval *et al.* [5] reported the ^{42}K efflux of "myoblast-enriched" primary cultures of neonate rat heart cells (grown as confluent sheets consisting of spontaneously beating muscle cells and fibroblastlike cells) in terms of multicompartmental kinetics which could not be correlated, however, with the apparent morphological simplicity of the preparation. Since the thinness of these preparations would tend to minimize problems associated with diffusional gradients in extracellular space, it is our opinion that cellular heterogeneity could perhaps have been a contributing factor to the multiple compartment kinetics.

The present study exploits the unique properties of a new preparation of growth-oriented heart cells [14] to gain insight into several problems created by heterogeneous cell types and extracellular space exchange in describing the transmembrane movement of potassium ions across cardiac cell membranes.

Materials and Methods

Tissue Culture

Heart cells oriented about a nylon monofilament were obtained by the methods described in the accompanying paper [14]. These preparations were obtained by trypsin disaggregation of 11-day-old embryonic chick hearts and consisted of spontaneously beating primary cultures of muscle and fibroblastlike cells as well as quiescent secondary cultures of fibroblastlike cells derived from primary heart cell cultures.

Potassium Efflux

Preparations were placed in a temperature and humidity controlled CO_2 incubator and equilibrated for 45 to 90 min at 37.5 °C in 2.5 ml of a radioactive loading solution. Potassium-42, obtained from New England Nuclear, Boston, Massachusetts or from Nuclear Energy Services, North Carolina State University, Raleigh, North Carolina was converted to an isotonic KCl solution and an appropriate amount was added to a balanced salt solution to obtain a final concentration of 5.4 mM K in the loading solution with an activity range between 5 and 10 μCi/ml. The modified Earle's balanced salt solution was of the following composition (mM): NaCl, 118; $NaH_2PO_4 \cdot H_2O$, 0.94; $MgSO_4 \cdot 7\,H_2O$, 0.81; $NaHCO_3$, 26.2; $CaCl_2$, 2.7; glucose, 5.6. Bovine serum albumin (Sigma) was neutralized to pH 7.4 with 1N NH_4OH and added to the solution at a final concentration of 7.0 gm/liter in order to obtain a protein concentration roughly equivalent to that of 10% whole serum [35]. The solutions were filter sterilized under pressure with 5% CO_2/air through either a membrane (0.45 μm, Millipore) or a cartridge (Ultipor URA, Pall).

At the end of the incubation period, the preparations were placed into a test tube containing 3.5 ml of potassium-free salt solution and transferred immediately (ca. 10 sec) to a constant temperature perfusion chamber (Fig. 1). Within 1 min after a rapid rinse, the preparation was stimulated electrically and perfused at a flow rate of 3.5 ml/min. The transfer and rinse solutions, together with the effluent samples which were collected in test tubes at 1 min intervals were subsequently counted in a well-type thallium-activated sodium iodide crystal (Model 1100, Nucleus). At the termination of an efflux experiment the preparation was quickly removed from the perfusion chamber and placed into a collecting tube which contained 3.5 ml of solution containing 118 mM KCl as a substitute for NaCl in order to equilibrate the remaining intracellular radioactive potassium. The amount of radioactivity remaining in the tissue was determined after equilibration for 1 hr in this solution. Correction for background counts and isotope decay were determined for each sample and the corrected data were processed to provide the efflux rate constant at the midpoint of each sample interval [2].

Evaluation of the Perfusion System

Temperature stability of the perfusion system was measured by a rapid response thermistor (Model 524, Yellow Springs Instruments) sealed into the flux chamber. The chamber temperature was constant over a 1-hr test period and was independent of flow rates up to 9.0 ml/min. The efflux rate constant of preparations equilibrated with potassium-42 was not significantly affected when the perfusion rate was changed from 1.3 to 7.0 ml/min. Therefore, to collect a sample volume equivalent to the capacity of the scintillation well during a 1-min interval, an intermediate perfusion rate of 3.5 ml/min was selected.

The time constant of the flux chamber was measured at a perfusion flow rate of 3.5 ml/min by two techniques. In one method, the perfusion system was filled with saline and the time course of the conductivity change between the chamber electrodes was measured as the saline exchanged with distilled water in the flux chamber. The time for a 95% response was 116 sec ($t_{1/2}$=27 sec). In the second method, perfusion was started immediately after a bolus of radioactive loading solution was injected into the top of the flux chamber, and efflux samples were collected and counted in the usual manner. The washout of activity was 95% complete in 30 sec, considerably faster than the electrically measured response. It is apparent that, in certain conditions, the time constant of the flux chamber would be the limiting factor in determining rapid components of ^{42}K efflux kinetics.

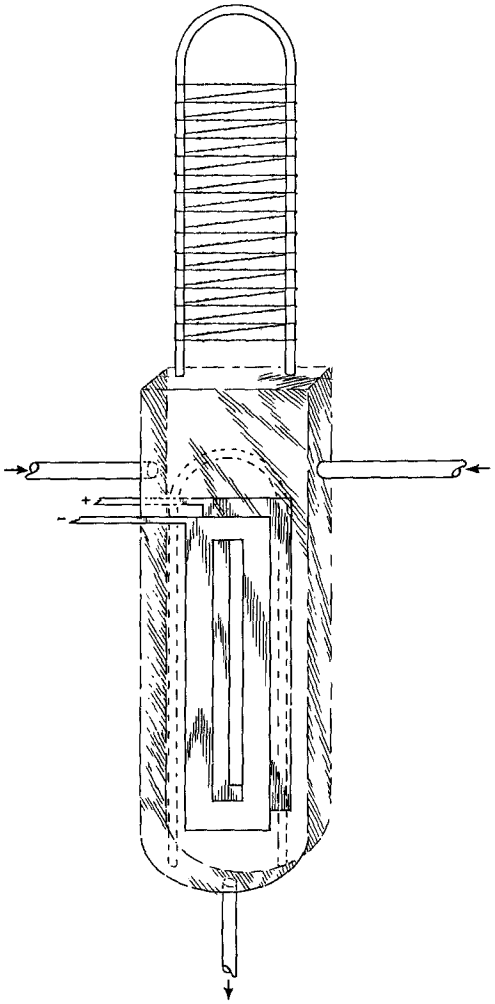

Fig. 1. Schematic representation of the preparation and flux chamber. Dotted lines indicate the location of the preparation between the parallel silver-plate stimulating electrodes (+, −) whose dimensions covered the thread-bearing areas of the preparation and the design of which enabled the monitoring of cellular contractions during the flux experiments by closed-circuit television microscopy (Model HV16S, Shibaden; Model X, Olympus; Model 115, Sony). Synchronization of the contractions in the preparations was accomplished by field stimulation with a pulse drive provided by a bi-phasic stimulator constructed in the laboratory. Perfusion fluid was directed through a heat exchanger into the chambers (*see* arrows) by a peristaltic pump (Model 1201, Harvard Apparatus) at a flow rate of 3.5 ml/min. Temperature control of 37.5 °C was provided by circulating water at a constant temperature (Model FGE, Haacke) through ducts which surrounded the channel housing the preparation. Perfusion fluid leaving the flux chamber (as indicated by arrow) was collected in test tubes mounted in an indexing carousel under the flux chamber

An approximation of this limitation is described by the empirical relationship [32]:

$$1/k_m = 1.05[(1/k_{ch})^2 + (1/k_{pr})^2]^{1/2}. \quad (1)$$

k_m is the observed rate constant, k_{ch} is the chamber rate constant, and k_{pr} is the preparation rate constant. According to this relationship, the flux chamber would be adequate (maximum error of 5%) for determining efflux rate constants up to 0.30 min^{-1}.

Experiments were designed to investigate whether a component of the efflux kinetics could be attributed to the preparation substrate. Nylon-bearing supports were equilibrated in radioactive loading solution, rinsed by medium for 5 sec, and then perfused in the efflux chamber. The efflux kinetics of the blanks were described by two components: a rapid rate constant similar to that of the flux chamber which could have represented surface adsorption or carry-over of loading solution, and a second component which exchanged at a very slow rate beyond the resolution of this system.

As a final check on the experimental technique, the potassium content of preparations subjected to a simulated experiment was compared with that of preparations maintained under control conditions in the culture incubator. The potassium content was analyzed by the method described in the accompanying paper [14]. The results supported the validity of the experimental design since the ratio of potassium per mg dry weight of the test preparations to the control preparations was 1.04.

Compartmental Analysis of Efflux Data

Compartmental analysis of the experimental efflux data was accomplished by fitting the data points with a curve defined by the sum of two exponential functions:

$$Y = IC_1 \exp(-k_1 t) + IC_2 \exp(-k_2 t). \quad (2)$$

There are four variables in this equation: IC_1 represents the initial conditions of compartment 1; k_1 represents the rate constant of compartment 1; IC_2 represents the initial conditions of compartment 2; and k_2 represents the rate constant of compartment 2. If none of these variables is known, no unique solution to the curve fitting the data can be found; however, by stipulating the values of one of the variables, a unique solution can be found. This was accomplished by setting the rate constant for the slow compartment equal to the rate constant obtained from preparations containing only fibroblastlike cells. To perform the actual curve fitting, the two-compartment equation was solved by an analog computer (Model 580 Analog/Hybrid Computing System, EAI) that was controlled by a digital computer (Model PDP-15, Digital Equipment Corp.) which provided X and Y coordinates and experimental data points. The data was then converted to analog signals (Model 693 Data Conversion System, EAI) and displayed on a large screen storage oscilloscope (Model 611, Tektronix). The digital computer alternately directed display of the analog solution, coordinates, and data points at a rapid repetitive rate, and between displays the analog computer automatically reset to initial conditions to permit the input of new variables (IC_1, IC_2, k_1). When a suitable curve could be fitted to the data points a hard copy unit (Model 4601, Tektronix) produced a record of the oscilloscope display.

Computational Solutions of the Radial Diffusion Equation

The equation for radial diffusion in a cylindrical muscle bundle [29] was solved by digital computation (PDP-11, Digital Equipment Corp.) using representative parameters obtained from previous experiments with embryonic chick hearts [4]. The variables in

the equation include membrane flux (mole $cm^{-2}sec^{-1}$), cell volume-to-surface area (cm), intracellular and extracellular potassium concentration (mM), tissue packing fraction, and radial diffusion time (sec). The solution of the equation involves the zeros of Bessel functions of the first kind, the first ten of which were used in the solutions reported in this study. Details of the Fortran computer program which contains parameters used in the solution and the natural logarithm of the relative intracellular specific activity as a function of washout time are described elsewhere [12].

Results

Potassium Efflux Kinetics of Contractile Preparations

The rate of potassium-42 efflux from contractile preparations, synchronously paced at 150 impulses per min declines continuously as a function of time after removal from the loading solution (Fig. 2). During the first 2 min, a large fraction of the decline in activity can be attributed both to transfer of the loading solution with the preparation and to an indeterminate exchange in extracellular space. Careful examination of Fig. 2 and calculation of the rate constants by the method of Caldwell and Keynes [2] shows the decline in the rate of efflux representative of multicompartment kinetics.

Fig. 3 shows the effect of an abrupt increase in the rate of stimulation from 150 to 250 impulses per min for a period of 5 min beginning at the 12th minute of ^{42}K efflux. In this experiment, the external potassium was reduced to emphasize the effect on the efflux rate which in 5.4 mM external potassium was barely detectable. Increasing the stimulation rate after the initial 30 min of ^{42}K efflux did not alter the efflux rate, and therefore suggested that slowing of the efflux rate with time in these preparations (*see* Fig. 2) might be accounted for by the presence of the inexcitable, fibroblastlike cells [14].

Potassium Efflux Kinetics of Noncontractile Preparations

Potassium efflux kinetics of growth-oriented preparations of secondary cell cultures which were unresponsive to electrical stimulation are shown in Fig. 4. These noncontractile preparations are characterized by a slow, stable efflux rate which, contrary to the contractile preparations, does not decrease with time during the efflux period. In five experiments, the rapid phase of ^{42}K exchange was complete in 2 to 5 min after which

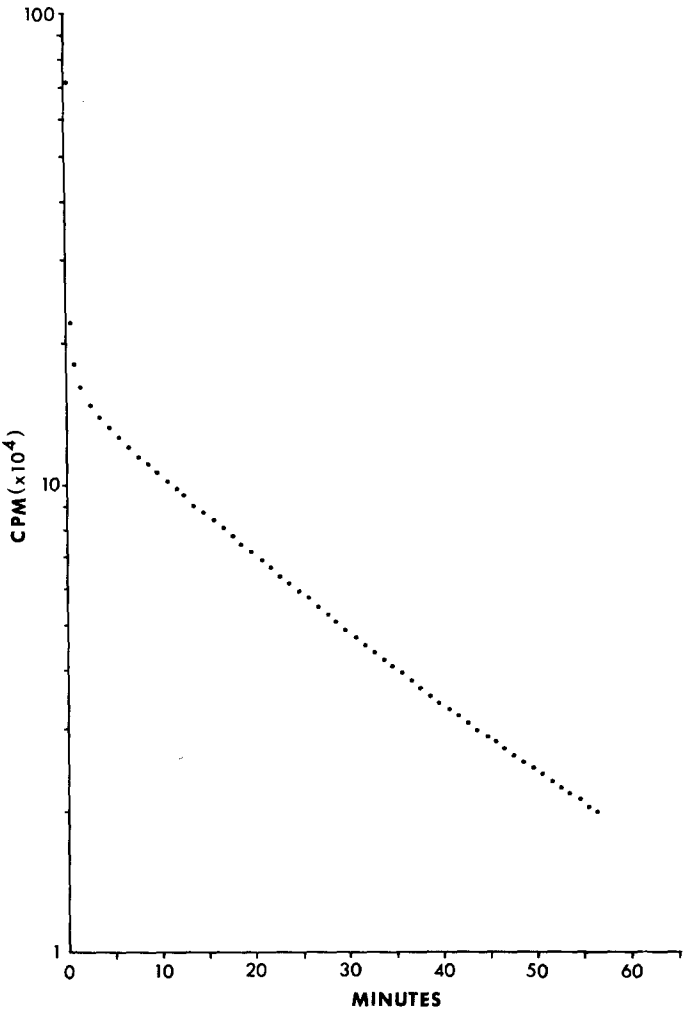

Fig. 2. Time course of potassium-42 efflux from a contractile preparation of growth-oriented heart cells that were synchronously paced at 150/min. Tissue radioactivity, corrected for isotopic decay and background is expressed on a logarithmic scale on the ordinate in counts per minute. Time (min) from the onset of perfusion with nonradioactive media containing 5.4 mM potassium at 37.5 °C is on the abscissa. The decline in the rate of efflux at long times is representative of a multicompartment system

time the kinetics were characteristic of a single exponential. The rate constant for these preparations was 0.015 ± 0.001 min^{-1} (mean $\pm$ SD). Electrical stimulation, at rates used to control the frequency of spontaneously beating preparations, had no detectable effect on the tracer kinetics of the noncontractile preparations.

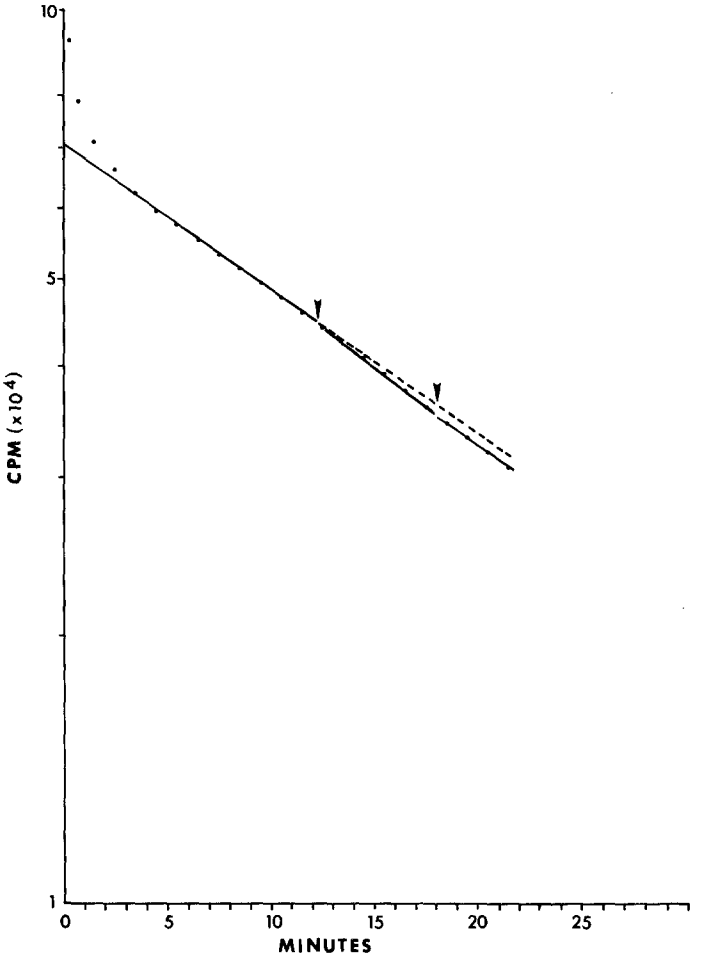

Fig. 3. Effect of stimulation rate on potassium-42 efflux kinetics of a contractile preparation in 2.0 mM external potassium. Temperature, 37.5 °C. Stimulation rate was increased from 150/min to 250/min between the arrows. Dashed line depicts the small deviation of the efflux rate in response to the increase in stimulation rate

Compartmental Analysis of Efflux Kinetics of Contractile Preparations

The previous study clearly showed the presence of fibroblastlike cells in the contractile preparations [14] and the present findings reveal the slowness of the ^{42}K efflux from these cell types when compared with that obtained from preparations comprised mainly of muscle cells. This evidence implicates a contribution of the fibroblastlike cells to the efflux kinetics of the contractile preparations and provides the basis for propos-

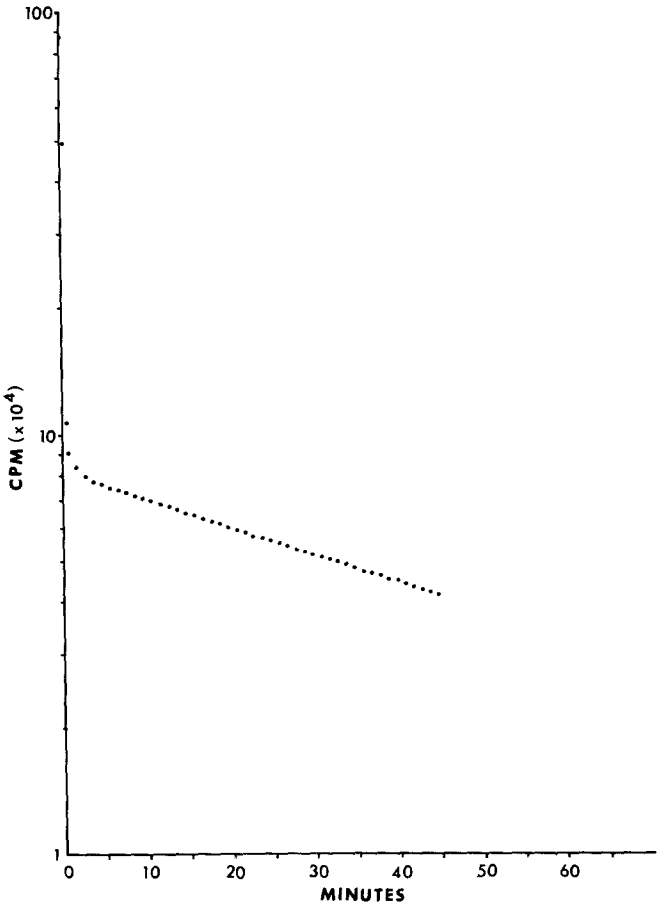

Fig. 4. Potassium-42 efflux kinetics of a noncontractile preparation in 5.4 mM external potassium at 37.5 °C

ing a two-compartment model to describe the efflux data obtained from contractile preparations. In this model, the compartments are assumed to be parallel, both exchanging directly with the bathing solution. The size of the compartments in the model were not fixed because the content of muscle and fibroblastlike cells in the preparations were observed, microscopically, to vary with different cultures. Furthermore, the preparations seldom remained in the ^{42}K loading solution for the period of time that would be required to fully equilibrate the fibroblastlike cells.

The efflux data were analyzed by analog curve fitting and, as shown in Fig. 5, were reasonably fit by the model. The rate constant, from

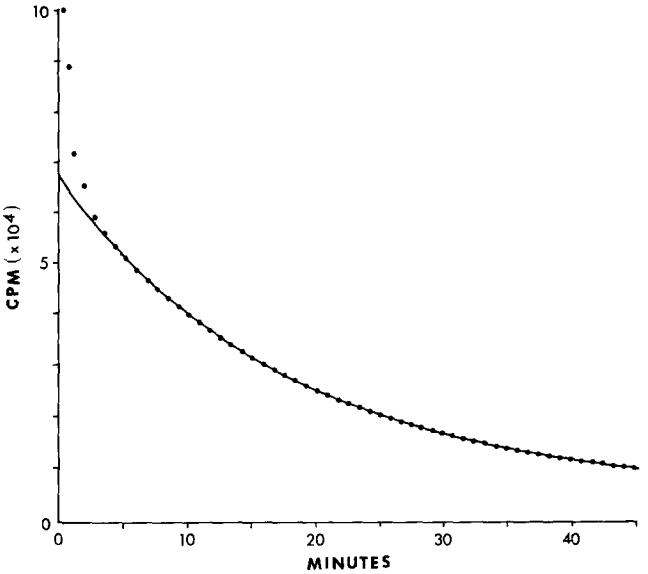

Fig. 5. Analog computer curve fit for potassium-42 efflux kinetics of a contractile preparation in 5.4 mM external potassium at 37.5 °C. Stimulation rate, 150/min. Analog parameters: Initial conditions of fast compartment (48,400 cpm) and slow compartment (19,000 cpm). Rate constant for fast compartment (0.062 min^{-1}) and slow compartment (0.015 min^{-1})

22 preparations, for the fast compartment was equal to $0.067 \pm 0.009\ min^{-1}$ (mean $\pm$ SD) and independent of preparation dry weight and time of loading. The size of the compartments, determined from the initial conditions of the analog model and corrected for time of preparation loading, corresponded to the content of muscle and fibroblastlike cells estimated from microscopic observations of contractile preparations from the same culture. The observed variation was more pronounced between different cultures than within preparations of a given culture.

Analysis of Diffusional Limitations on ^{42}K Efflux

Extracellular diffusion delays can create significant errors in isotopic rate constants because tracer exchange from multicellular preparations will be slowed by the recycling (reflux) of the tracer into the cells [17, 20, 28]. Recently, a general equation for studying tracer diffusion in cylindrical muscle bundles was derived and applied to cardiac muscle [29]. In the present study, digital computational methods were used to

solve the radial diffusion equations for several important variables which can affect isotopic reflux, namely, transmembrane flux, cell volume-to-surface area, and cell packing fraction.

Five values of transmembrane potassium flux were selected within the range of 5–100 pmoles $cm^{-2}sec^{-1}$ and applied to the solution of the radial diffusion equations. The apparent (or measured) flux was then plotted as function of the radial diffusion time, a parameter equal to the square of the preparation radius divided by the extracellular diffusion coefficient. In Fig. 6*A*, it is evident that with higher values of radial diffusion time, true values of transmembrane flux which differ by as much as a factor of 100, would become experimentally indistinguishable. Experimentally, values for diffusion time are influenced by the diameter of the preparation and by the diffusion coefficient of the tracer in extracellular space, a factor which can be modified by the macromolecular composition of the extracellular matrix in cardiac muscle [30]. The diffusional limitations are clearly demonstrated if the percentage error by which the experimentally determined flux underestimates the true flux values is plotted with respect to the radial diffusion time (Fig. 6*B*). Thus, in a given preparation, a lower value of the true flux will reduce the error in measurement of that flux. Indeed, when the steady-state potassium exchange was measured from preparations of cat papillary muscle at several values of temperature and plotted as a function of preparation diameter [33], the magnitude of the measured flux obtained at 37.5 °C was inversely related to the diameter of the preparation. However, at lower temperatures, the low values obtained for the measured flux were less sensitive to changes in preparation diameter, a finding which can be predicted from the radial diffusion equation illustrated in Fig. 6*A*.

For a given value of membrane flux, the ratio of cell volume to surface area (V/A) is a second significant factor in determining the magnitude of the radial diffusion error. A cell with a small volume-to-surface area and a transmembrane flux equivalent to a cell with a large V/A must turn-over cellular potassium at a proportionately higher rate. Consequently, the more rapid tracer exchange would increase the specific activity of the extracellular space and promote tracer reflux. Therefore, as illustrated in Fig. 6*C*, differences in V/A can have a marked effect on the percentage error of the measured flux.

A third significant determinant of radial diffusion error is the density of cells (packing fraction) within a preparation. Clearly, ions will exchange less readily with the perfusion solution in a tissue comprised of tightly packed cells than a loose cellular network. As shown in Fig. 6*D*,

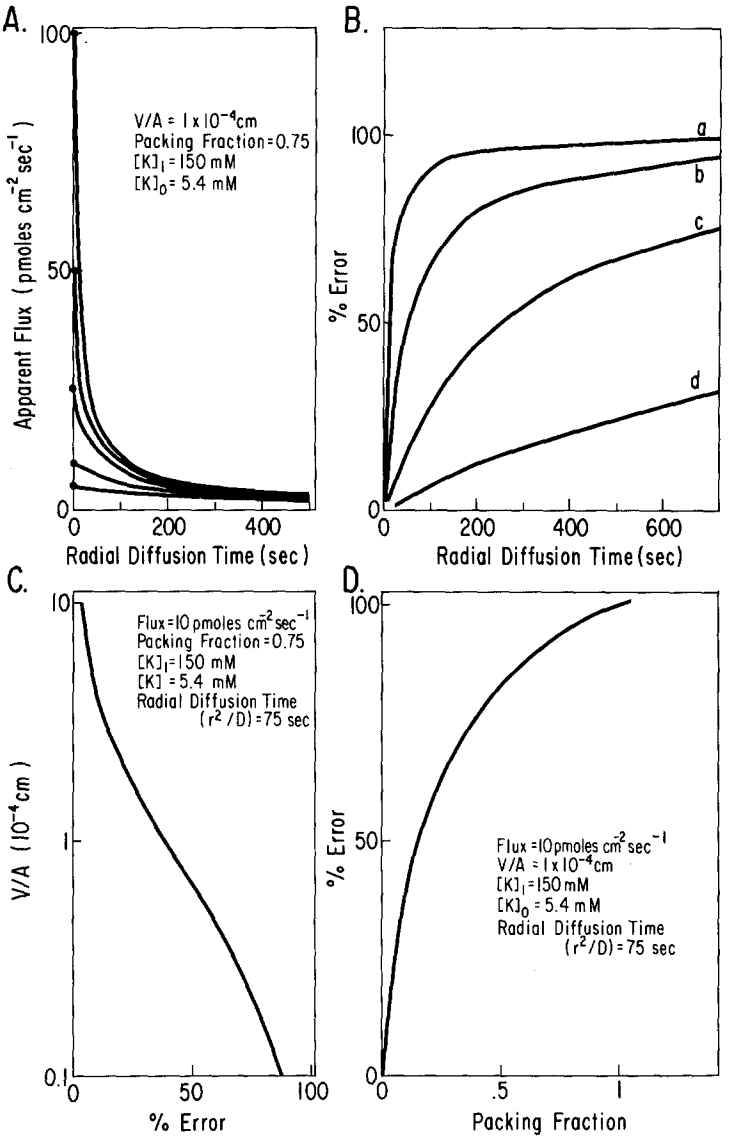

Fig. 6. Graphical representation of computational solutions of the radial diffusion equation. (*A.*) Apparent potassium flux as a function of radial diffusion time, Tr, described by the square of the preparation radius divided by extracellular diffusion coefficient (r^2/D). The true membrane flux values for each curve are shown at Tr=0. (*B.*) Percentage error of measured flux values (pmoles cm^{-2} sec^{-1}) of (*a*) 100 (*b*) 25 (*c*) 5 (*d*) 1. Parameters used in the computations are the same as those noted in Fig. 6*A*. (*C.*) Percentage error of measured flux as a function of cell volume-to-surface area (V/A) ratio. (*D.*) Percentage error of measured flux as a function of cell packing fraction

an increased packing fraction will markedly increase the percentage error of the measured flux.

Discussion

The emphasis of this study was threefold: to determine the extent to which muscle and fibroblastlike cells in a newly developed preparation of growth-oriented heart cells [14] influence ^{42}K tracer kinetics; to resolve and identify the components which contribute to extracellular diffusional delays in multicellular preparations; and ultimately to accurately determine the magnitude of potassium-42 efflux across cardiac cell membranes.

Analysis of ^{42}K Efflux Kinetics

Growth-oriented strands of embryonic heart cells provide a means for separating and identifying the contributions of heterogeneous cell populations to tracer kinetics. The results show that tracer kinetics in a homogeneous preparation of quiescent, fibroblastlike cells are well described by the mathematical formulations for a single compartment which exchanges with a well-stirred perfusing solution. In contrast, contractile preparations, which contain two populations of cells, demonstrate efflux kinetics that are often reported for naturally occurring preparations of cardiac muscle. By compartmental analysis, it was possible to resolve the tracer kinetics into two components, each representative of the two cell types in the growth-oriented strands; namely, a slow component associated with fibroblastlike cells and a fast component associated with cardiac muscle cells.

An equivalent analysis has not been possible for naturally occurring preparations of cardiac muscle because of the thickness of the preparations and the presence of vascular elements (capillaries, vascular smooth muscle, blood cells), specialized cardiac conducting cells and a substantial amount of extracellular material which impedes the investigator from isolating the kinetics of the cardiac cell membrane. In addition, the electrochemical gradients in isolated preparations of naturally occurring cardiac muscle can be widely variable. For example, Langer and Brady [25] reported a 50% decrease in intracellular potassium in their isolated preparations of ventricular muscle in the 4-hr period following its isolation. These combined difficulties no doubt account for the fact that

ionic permeabilities of naturally occurring cardiac muscle determined from ionic flux studies have not correlated with those determined from electrical measurements [3, 11].

The results from previous investigations with both ordinary and tissue-cultured preparations of embryonic heart can be related to those obtained for the fibroblastlike cells in the present study. Burrows and Lamb [1] reported a value of 0.026 min^{-1} for secondary cultures of noncontractile cells from embryonic chick hearts, which is of the same order as that of 0.015 min^{-1} for the noncontractile preparations in this study (the small differences may reflect differences in the method for obtaining the secondary cell cultures). Isolated preparations of 12 to 17 day embryonic hearts [4] showed multicompartment efflux of ^{42}K during the initial 30-min period, declining to a steady value equal to that of the fibroblastlike cells in the present study. Furthermore, Cheneval *et al.* [5] reported a mean half-time of 38.4 min ($k=0.018$ min^{-1}) for the potassium efflux of fibroblasts derived from neonatal rat hearts.

Table 1 was compiled to compare the results of ^{42}K efflux kinetics for muscle cells in this study with representative studies from various species of cardiac muscle. Attention must be drawn to the fact that many of the studies cited had been analyzed as if the preparations were describable by single compartment kinetics, even though a multicompartment analysis would have been the more appropriate. It is evident that most preparations of vascularly perfused heart muscle appear to have slow, single compartment kinetics when perfused *in vivo* by the systemic circulation or *in vitro* at rates below about 5 ml/min/g of tissue. In such cases, tracer exchange could have been limited by the rate of perfusion [34]. In addition, it is quite likely that the nonvascularly perfused preparations, which exhibit single compartment kinetics, may have been severely limited by radial diffusion.

It is difficult to compare the two-compartment kinetics of the present study with that of naturally occurring preparations of cardiac muscle because factors other than differences in cell type can give rise to multicompartment kinetics in such preparations. For example, in vascularly perfused tissues, if the perfusion fluid is not uniformly distributed throughout the preparation, the less adequately perfused regions will appear as a slowly exchanging compartment. Alternatively, in nonperfused preparations, differences in preparation geometry can affect radial diffusion and promote the appearance of multicompartment kinetics. Furthermore, in both preparations, cell injury associated with the dissection and metabolic deficiencies resulting from inadequate tissue perfusion

Table 1. Potassium-42 efflux kinetics of cardiac muscle preparations

Preparation (species)	Perfusion (type-rate)	Efflux kinetics	Rate constants		Ref.
			fast (min^{-1})	slow (min^{-1})	
(Rabbit)					
Heart	VP-(5.0 ml/min/g)	MC	0.115	0.013	16
Atria	BP	MC	0.050	0.012	38
Ventr. Septum	VP-1.5 ml/min/g	SC	–	0.011	26
(Guinea Pig)					
Heart	VP-(5.0 ml/min/g)	MC	0.087	0.010	40
Ventricle	BP	(SC)	–	0.016	6
(Rat)					
Ventricle	VP-2.5 ml/min/g	SC	–	0.021	41
Ventricle	VP-20 ml/min/g	MC	(0.070)	(0.030)	34
Ventricle	BP	(SC)	–	0.027	6
Atria	BP	SC	–	0.015	8
TC-Heart	BP	MC	0.070 (mean)	–	5
(Mouse)					
Heart	VP-(3.3 ml/min/g)	SC	–	(0.028)	15
(Cat)					
Papillary	BP	SC	–	0.010	7
Papillary	BP	MC	NA	0.012	36, 37
(Dog)					
Heart	VP-Systemic	SC	–	0.008	9
Heart	VP-0.5 ml/min/g	SC	–	0.018	43
Ventricle	VP-1.0 ml/min/g	(SC)	–	0.014	25
Purkinje	BP	MC	NA	0.011	36, 37
(Sheep)					
Purkinje	BP	MC	NA	0.008	3
(Chick embryo)					
Ventricle 7 day	BP	MC	NA	0.017	21
Ventricle 7 day	BP	MC	NA	0.015	4
TC-Fibroblast	BP	SC	–	0.021	1
TC-Heart	BP	MC	0.067	0.015	PS
(Frog)					
Atria	BP	MC	NA	0.027	10
Ventricle	BP	SC	0.082	–	22
Ventricle	BP	MC	0.065	0.009	39
Heart	BP	MC	0.036	0.006	39

BP = Bath perfusion
VP = Vascular perfusion
MC = Multicompartment
SC = Single compartment
NA = Not available
TC = Tissue cultured
PS = Present study
Values in parentheses were estimated from reported data.

may produce a population of cardiac cells with different potassium exchange characteristics.

Determination of Potassium Efflux from Cardiac Muscle Cells

The rate constant for potassium efflux (k) obtained in the present study, together with the previously described determinations [14] of internal potassium concentration (C_K) and the ratio of cell volume to surface area (V/A) provide the basis for calculating the absolute value of the potassium efflux (J_K) from the relation [20]:

$$J_K = kC_K \text{V/A}. \tag{3}$$

The potassium efflux of cardiac muscle cells is 15.7 pmoles $cm^{-2}sec^{-1}$ whereas the value obtained for the fibroblastlike cells is 1.88 pmoles $cm^{-2}sec^{-1}$, the eightfold difference reflecting the higher rate constant and V/A of the muscle cells. A comparison of the ^{42}K efflux values in the present study with those previously reported for naturally occurring cardiac muscle is of little merit because of the problems associated with the tracer measurements (as described above) and the assumptions used in flux calculations when there was a lack of quantitative data. For example, Rayner and Weatherall [38] reported that the potassium efflux in quiescent rabbit auricle was equal to 16.9 pmoles $cm^{-2}sec^{-1}$. Although this value appears to correlate well with the present study, calculation of the potassium efflux included a correction factor of 2 to 2.5 for diffusion delays [20].

It is useful to compare the value of potassium efflux for cardiac muscle determined in this study with that reported for nerve fibers of small diameter (30 μm) under well-controlled conditions [19]. Although values for potassium efflux in nerves were obtained at a stimulation rate of 300 min^{-1}, it is possible to recalculate the ^{42}K efflux at a rate of 150 min^{-1} (present study) from the reported extra efflux per action potential. In this manner, an efflux of 26.0 pmoles cm^{-2} sec^{-1} can be calculated in nerve as compared to the value of 15.7 pmoles cm^{-2} sec^{-1} for cardiac muscle at the same stimulation rate.

Attempts to determine effects of stimulation on ^{42}K efflux from the tissue-cultured cardiac muscle cells, using the method of Keynes [20], are complicated by two factors: spontaneous activity of the preparations precludes a determination of the resting flux levels and the maximum rate of stimulation is limited to 300/min. Thus, measurements

of changes in ^{42}K efflux are restricted to relatively narrow changes in rates of stimulation. In the present study, an estimate of the effects of an increase in rate of stimulation from 150/min to 250/min (*see* Fig. 3) caused the efflux rate constant to increase by only a factor of 1.11. Applying this increase to the mean value of the rate constant of muscle cells determined by compartmental analysis results in an extra flux per impulse of approximately 1.0 pmole cm^{-2} sec^{-1}. This value is relatively close to the flux of 1.5 pmoles cm^{-2} sec^{-1} (calculated from the membrane capacitance of 1.54×10^{-6} F cm^{-2} [27]) which would be required to repolarize a cardiac action potential of 100 mV amplitude. The slight difference in flux values obtained by the two methods should not be taken as evidence to support the involvement of other ionic currents in the repolarization of the cardiac action potential because of the errors introduced when determining changes in rate constants by the multicompartment kinetics. Since discontinuities are produced in the data when the stimulation rate is changed during an experiment, and curve fitting requires uniform data, experiments involving rate changes cannot be readily analyzed by compartmental methods.

Evaluation of Diffusional Limitations in Growth-Oriented Heart Cells

A major difficulty in directly applying the computed solutions of the radial diffusion equation [29] to the present experimental results as well as to those of other studies is concerned with the conflicting values reported for the diffusion coefficient in extracellular space. Weidmann [42] attempted to measure the longitudinal diffusion of potassium-42 in trabecula muscle and obtained a value of 7.9×10^{-6} cm^{-2} sec^{-1} which was shown subsequently to represent a radial diffusion coefficient of approximately 2.3×10^{-6} cm^{-2} sec^{-1} [29]. Depending on the true transmembrane flux, it was noted that the measured value for the longitudinal diffusion coefficient could have been overestimated by a factor of 4 to 16. From measurements of radial diffusion in rat skeletal muscle under conditions that precluded interference from transmembrane flux, McLennan [31] calculated a diffusion coefficient of 4.75×10^{-6} cm^{-2} sec^{-1}, a value close to the estimate of 4.5×10^{-6} cm^{-2} sec^{-1} in frog skeletal muscle [20].

The value of the extracellular diffusion coefficient of tissue-cultured preparations of heart cells is probably closer to the value in free solution than in other preparations because of the relatively sparse extracellular matrix. As a conservative estimate, the value of McLennan [31] was

used to calculate a mean radial diffusion time (r^2/D) of 1.9 sec for the growth-oriented heart cells ($r=30$ μm).[1] In contrast, a preparation of 1 mm diameter would have a calculated radial diffusion time of 590 sec. Using this estimate, it would be possible to measure fluxes as high as 100 pmoles cm^{-2} sec^{-1} from the nylon-supported preparation with an error of less than 10% (Fig. 6*B*).

In addition to the extreme thinness of the nylon-supported preparation, other advantages are present which would minimize the effects of radial diffusion, namely the relatively low packing fraction of 0.75 [27] and the absence of transverse tubules.

In conclusion, the present study illustrates that radioisotopic flux analyses can be successfully applied to tissue-cultured preparations of growth-oriented heart cells. The unique geometry of the preparation was an essential factor in enabling the experiments to reveal the extent to which cellular heterogeneity and tracer diffusional limitations can alter the determination of true transmembrane fluxes. Of equal importance, the preparations have provided the basis for a series of experiments (currently in progress) which are designed to test whether a correlation exists between the value of membrane conductance determined from potassium flux measurements and that derived from electrical measurements.

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1 The nylon-supported preparation is actually an annulus of cells with an impermeable core rather than a cylindrical bundle of cells. Since the radial diffusion time was calculated on the basis of the overall diameter, the value of radial diffusion time is overestimated.

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