Some Cation Interactions in Muscle

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ABSTRACT It has been possible to treat potassium, rubidium, and cesium ion entry into frog sartorius muscle by the use of a model which assumes a limited number of sites at the cell surface. The ion concentration in an outer surface layer is regarded as the main factor determining the rate of inward movement. It is supposed that the concentration of ions in the external solution is effective in promoting inward movement only to an extent determined by the fraction of sites occupied. Equations are derived from the model which fit the inward flux *versus* applied concentration curves experimentally determined for the three ions. The ions were found to compete for the postulated sites in various bi-ionic mixtures, the competition being satisfactorily described by equations derived from the model. The constants assigned to each ion remain invariant and independent of gradients in electrochemical potential. The order of decreasing exchange rate found is K > Rb > Cs. The order of decreasing site affinity found is Rb > K > Cs which is the same order as that observed for the ion selectivity deduced from analytical measurements of cation preference after equilibration in various equimolal mixtures (Lubin and Schneider (21)). The manner in which such a model might affect the application of a theory which assumes electrical driving forces as well is discussed.

INTRODUCTION

In a previous study it was found that the influx per unit of applied concentration declined in frog muscle for rubidium and cesium ions as well as for potassium ions when the concentration was raised (Sjodin (1)). Since these ions depolarize the muscle cell membrane as the external concentration is elevated, a possibility exists that a decline in the electrochemical potential gradient for inward ion movement is responsible for the declining ratio of influx to concentration. One difficulty in testing this hypothesis is the lack of a physical model for the membrane which could provide a measure of the electrochemical driving forces acting on ions with a high degree of certainty. It was hoped that the constant field equation employed by Goldman (2) and by Hodgkin and Katz (3) would provide at least an estimate of the effect of the membrane potential on inward ion movement. It was found that this

equation could account for the decline in flux to concentration ratio for potassium ions but not for rubidium and cesium ions. For the latter ions the ratio declined more with rising concentration than predicted by the constant field theory. The discrepancy could be explained on the basis that the number of membrane sites for rubidium and cesium ions became a limiting factor at higher concentrations. Another observation in this work was that the potassium fluxes were lowered by the presence of either rubidium or cesium ions. Calculations using the constant field equation indicated that the permeability coefficient for potassium ions was lower in the presence of rubidium or cesium ions and appeared to be a function of the concentration of these ions. A major difficulty with the application of this theory was that the number of membrane sites for potassium ions did not appear to be limiting at high potassium concentrations and yet rubidium and cesium ions appeared to compete with potassium ions for available membrane sites.

One generalization appears to be quite evident from this work. The influxes of potassium, rubidium, and cesium ions are not independent and hence the independence principle used to derive the constant field equation and similar equations cannot be applied with any assurance that correct results will be predicted. Though potassium influx can be fitted to the constant field equation under approximately steady state conditions, Hodgkin and Horowicz (4) find that the potassium permeability coefficient varies with the electrochemical potential difference for potassium ions whenever it assumes a value different from zero. Ample evidence seems to exist, therefore, that the constant field equation does not provide a correct measure of the driving forces for ion movements in muscle cells (Sjodin (1), Mullins (5), Harris and Sjodin (6), Hodgkin and Horowicz (4), Adrian (7)).

The present work represents an attempt to find a more suitable model to describe passive cation movements in the muscle cell. The point of departure is the finding in the previously mentioned work (Sjodin (1)) that rubidium and cesium ions appear to compete with potassium ions for available membrane sites. Ling (8) has previously demonstrated potassium and rubidium ion competition in muscle and has pointed out that the potassium flux in muscle deviates from Fick's law in a way mathematically describable on the basis that a limited number of occupied sites determines the exchange rate. In addition, many workers have found it possible to describe ion movements in a variety of living cells by using the limited site model (Streeten and Solomon (9), Shaw (10), Glynn (11), Epstein and Hagen (12), Epstein (13), Harris and Sjodin (6)).

The experimental technique employed is the measurement of the uptakes of tracer potassium, rubidium, and cesium ions at a variety of concentrations of these ions and in a variety of mixtures. The advantage of treating all three ions together is that a more rigorous test of theory is obtained. With three

ions, for example, nine different combinations of tracer flux with non-tracer flux are possible. All nine combinations are successfully treated without a change in assigned constants.

METHODS

Experimental Methods All experiments were pertormed exclusively upon sartorii from *Rana temporaria*. The standard Ringer's solution used had the following composition: NaCl 80 mM, $NaHCO_3$ 30 mM, $CaCl_2$ 2 mM, KCl 2.5 mM or an amount specified in the text. Potassium, rubidium, and cesium ions were added as the chloride salt unless otherwise stated. Potassium ions were added in addition to and not by substitution for sodium ions. With rubidium and cesium ions some substitution for sodium ions was necessary for maintaining a constant muscle weight. Muscles were found to maintain their weight satisfactorily in solutions with a high rubidium concentration when 75 per cent of the RbCl was added in addition to and 25 per cent by substitution for NaCl. With CsCl, 50 per cent was substituted for NaCl. No significant weight changes occurred in the experiments reported except in the cases in which rate of swelling was determined in solutions of low sodium concentration. All solutions were made up freshly before use and were bubbled with a 95 per cent O_2–5 per cent CO_2 gas mixture during the experiments. The radioactive isotopes used were: K^{42}, Rb^{86}, Cs^{137}, and Na^{24}. Uptake rates were obtained by placing the muscles in solutions of known specific activity for varying periods of time and then counting the radioactivity in the carefully blotted muscle. Counting was effected by placing muscles beneath a Geiger counter in the case of potassium and rubidium ions. Cesium ions and, in some cases, potassium ions were assayed by counting in a well type scintillation counter. In all cases muscles were digested in 1 N nitric acid at the termination of each experiment. The 1 ml. digest was then diluted to 5 ml. and the solid material remaining centrifuged to the bottom. A sample of this diluted digest was then counted and compared with the count of an equal volume of soak solution. The samples were later used for flame photometry of the ion contents of the muscles. In the cases in which sodium output was followed, muscles were equilibrated overnight in a Na^{24} Ringer solution of high specific activity. Efflux was then determined by collecting samples into successive small volumes (1 to 5 ml.) of Ringer's solution of given composition. All experiments were carried out at 21° ± 1°C. and at a pH of 7.3.

All uptake rates determined are expressed per unit of wet weight of the whole muscle with no corrections whatever applied. Weighings were carried out as follows. A clean piece of filter paper was moistened with Ringer solution so as to be just saturated with fluid. The freshly dissected muscles were equilibrated in the Ringer solution for 15 minutes prior to weighing. Muscles were then carefully blotted on the moist filter paper to drain off any excess adhering solution. The manner of blotting did not cause any significant drainage of the extracellular space. To assess the experimental error involved in the weighings, two muscles were each weighed ten successive times, in every case being returned to the solution between weighings. The standard deviation of such a series of weighings was found to be ± 2 per cent.

Fluxes in whole muscle are lower than in single cells from the same muscle due to diffusion in the extracellular space, the so called "diffusion effect," (Keynes (14), Harris and Burn (15)). Sjodin (1) used the single cell data of Hodgkin and Horowicz (4) to estimate the diffusion effect factor for a large (100 mg.) muscle. The factor was about 1.5 and it is likely that the factor would be only about 1.2 for a smaller muscle (30 mg.). Thus if muscle weights varied between these extremes a source of variation

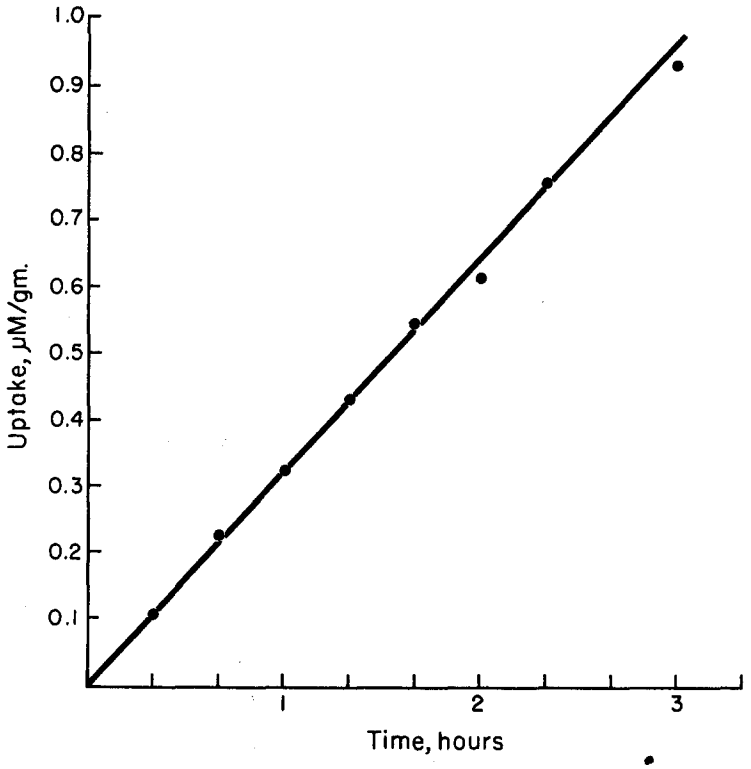

FIGURE 1. Experimentally determined rubidium uptake points are plotted against time. The Ringer solution used contained rubidium ions at a concentration of 0.2 mM and contained no potassium ions. The straight line drawn through the experimental points has a slope of 0.325 μmole per gram per hour.

of up to 1.3 could occur for no other reason. It would obviously be desirable to use only muscles in a very narrow weight range. For practical reasons the range selected in this work was 60 to 90 mg. The variation within this range is probably within the range of experimental variation arising from undetermined factors.

Method of Treating Data The influx of an ion into a cell is unambiguously determined both conceptually and experimentally as long as the uptake of tracer used is linear with time. The influx is most accurately determinable experimentally at very low concentrations when equilibration is slow and uptake of tracer is linear over a period of several hours. This situation is illustrated in Fig. 1 where rubidium

uptake is plotted when the external concentration of rubidium is 0.2 mM. The straight line obtained extrapolates through the origin to within experimental error because the proportion of the entering ions which resides in the extracellular space is very small.

As the external concentration is raised, tracer equilibration becomes more rapid and the time interval over which the uptake is linear with time becomes shortened. Since, for practical reasons, a smaller number of experimental points are available

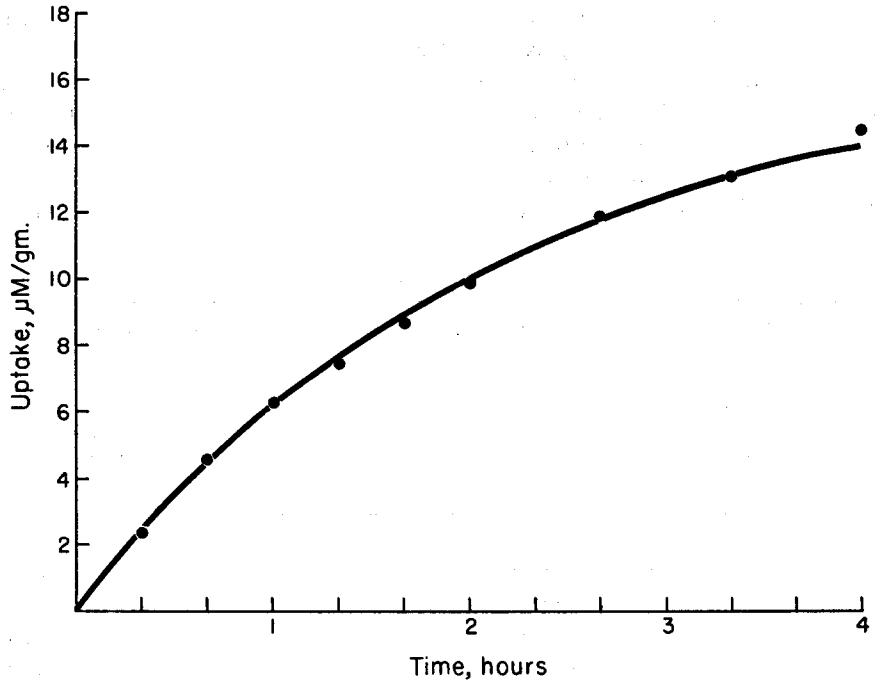

FIGURE 2. Experimentally determined potassium uptake at an external concentration of 2.5 mM is plotted against time in contact with the solution. The smooth curve fitting the points is a plot of the equation $C_i(t) = C_i(\infty)(1 - e^{-kt})$ in which $C_i(\infty) = 15.8$ μM/gm. and $k = 0.5$ hr.$^{-1}$

in the linear region as the concentration is elevated, it is important to know something of the kinetics of the uptakes. A plot of potassium uptake is presented in Fig. 2 for the case in which the external concentration is 2.5 mM. The smooth curve fitting the experimental points is a plot of a single exponential equation having the form $C_i(t) = C_i(\infty)(1 - e^{-kt})$. This is the equation that would be expected to govern the equilibration of tracer with a single cellular compartment bounded from the external solution by a thin resistive membrane whose permeability to the ion traced and whose membrane potential do not vary during the uptake experiment. For the data in Fig. 2 the constants required for a fit were $k = 0.5$ hr. $^{-}1$ and $C_i(\infty) = 15.8$ μM/gm. The analytical potassium content of this muscle at the end of the experiment as determined by flame photometry was 80 μM/gm. Evidently the tracer

appears to be equilibrating with a compartment containing about one-fifth of the muscle potassium. If uptake is followed beyond a 4 or 5 hour period at this concentration, the uptake points will be found to deviate from the exponential equation given. The deviation is slow and after a whole day of uptake the exchange might be 28 per cent instead of the initial 20 per cent. In this particular experiment one could account for the apparent incomplete exchange by assuming that the permeability to potassium of about 75 per cent of the muscle fibers was of an order of magnitude lower than that of the remaining 25 per cent. This is a very unlikely explanation, however, since the apparent size of the compartment with which tracer ions equilibrate increases markedly as the external concentration is raised. This is shown by the data for potassium ions given in Table I where the rate constant and concentration required in the equation are given at different concentrations of potassium in the solution. The aim of this work is not to explain these findings which have been previously dealt with in more detail by Harris (16). The aim is rather to obtain uptake rates from experimentally measured uptake points. Since the point of de-

TABLE I

External potassium concentration	Rate constant k $hr.^{-1}$	Inside concentration $C_i(\infty)$
mM		μM/gm.
2.5	0.5	15.8
5.0	0.5	25.0
10.0	0.5	44.0
15.0	0.5	60.0

parture has been taken as a concentration at which uptake is linear with time over the entire uptake interval of several hours, one can use the technique of exponential fitting at the higher concentrations to estimate the initial linear slope. The initial flux is simply given by the product of the rate constant and the concentration constant used in the exponential. There can be no doubt that the single exponential applies to the early linear portion of the uptake since it gives a satisfactory fit to data for a period of hours at low concentrations (<10 mM). The technique allows one to use the data from many uptake points over a time interval of hours and avoids the errors introduced by using very much fewer points in the linear interval.

As the external concentration is raised, an increasing fraction of the entering ions will reside in the extracellular space which will consequently become an increasingly important factor at the higher concentrations. The problem of the extracellular space is also relevant in expressing fluxes on a muscle weight basis. Tasker *et al.* (17) report a dispersion in measured extracellular space in sartorius muscle of from a space value of 8 per cent to one of 40 per cent in animals from different batches. These workers also report, however, that the spaces tended to be rather uniform in certain batches of animals. The latter finding was substantiated in this work as well as the former. It was found, for example, that fluxes determined per unit of uncorrected wet weight on a series of frogs from the same batch showed rather low standard deviations and also rather similar extracellular spaces as measured by the extrap-

olation technique to be discussed. The lowest standard deviation observed in a series of flux measurements on frogs from the same batch was ± 4 per cent. The highest standard deviation observed in such a series ot measurements was about ± 15 per cent. An average standard deviation obtained from several such series was about ± 7 per cent. If results from such a batch of frogs were compared with similar measurements on another batch obtained in a different season, for example, the agreement was often rather poor with variations up to 30 or 40 per cent. In such cases the lower flux values were always correlated with a high value for the extracellular space. One could obviously correct the whole wet muscle weights by factors obtained from estimates of the extracellular space to reduce the interbatch source of variation. This was not done for two reasons. The method used to estimate the space size was not absolutely accurate and may include a space accommodating some adsorbed ions. Second, muscles with widely varying extracellular space sizes may show different diffusion effect factors which would be difficult to estimate. A sounder procedure seemed to be to use frogs from a batch which did not show wide variations in flux values when the flux was expressed per whole muscle weight. The procedure could be checked by comparing the experimentally determined extracellular space estimates. They were found in such cases to show the sort of variation that would be consistent with the variation in flux. For example, consider two muscles each weighing 80 mg. one of which has an extracellular space of 20 per cent while the other has a space of 28 per cent. The corrected weight of the first muscle is 64 mg. while that of the second is 58 mg. The variation in flux caused by just the space variation would be about 10 per cent. The measured fluxes in such an instance would be found to lie within 10 per cent of each other. With this amount of variation it was not possible to improve the reproducibility of fluxes by applying the extracellular space correction. This must mean that the amount of space variation cited in the example would lead to a source of variation which could not short of an exhaustive statistical analysis be differentiated from the normal variation resulting from measurement errors and undetermined sources of variability. Applying space corrections, on the other hand, did not lead to greater variation in the fluxes. The procedure adopted was to apply no space correction to the muscle weights and to control the experiments carefully with paired muscles. The control in a competition experiment, for example, would be the flux of traced ions at the same concentration in the absence of competitor ions.

When the fraction of entering ions located in the extracellular space becomes significant, the origin for the kinetics of cellular uptake will be shifted to some point on the uptake axis. Strictly speaking, cellular penetration will begin the instant tracer ions reach the cell surface and standard two-compartment kinetics would be required for a rigorous treatment. This was not done since the time constants for cellular uptake indicated that extracellular space equilibration is about sixty times more rapid than cellular equilibration. In addition, one cannot be certain that some adsorption at the cell surface does not take place which would require a third compartment. In view of the rapidity of these processes compared to cellular uptake, the technique of extrapolation to the uptake axis would appear to provide a reasonable estimate of the extracellular space. The first few experimental uptake points (taken

increasingly close together as the concentration is raised) were found to be linear to within experimental error. A straight line extrapolated to the uptake axis yielded estimates of the extracellular space lying between extremes of 20 and 40 per cent in general agreement with the results of Tasker *et al.* (17) considering that frogs were from the same or from similar batches. The bulk of the values found were in the range from 26 to 35 per cent. These values indicate an average which is somewhat higher than the average of inulin spaces reported by Tasker *et al.* This tends to suggest that adsorption at the cell surface does occur. In any event, the quantity sought is the rate of movement into the cells. This should be assessed after outer regions have been largely equilibrated. Moving the origin to the extrapolation point on the uptake axis yielded curves for single ion movements (no competitor ions of another species) which could be fitted by the exponential equation previously given.

The procedure outlined for single ion movements became complicated when competing mixtures of ions were employed. When rubidium or cesium ions were used as competitors and an ion of a different species was traced, the uptake deviated from the single exponential equation after about an hour. The deviation was always in the direction of uptakes becoming lower. This behavior would be expected if the exchange rate between the ion traced and intracellular rubidium or cesium ions were different than that between traced ion and intracellular potassium ions. That this explanation is correct could be tested by tracing uptake into a muscle previously loaded with rubidium or cesium. It was found that a competitor ion of another species exchanged more slowly with intracellular Rb or Cs ions than with intracellular K ions. The kinetic anomaly mentioned, then, is to be expected. To obtain the initial penetration rate in such cases, uptake points in an early interval were made close together and the initial slope takenas the rate.

RESULTS

Theoretical The model employed to account for the experimental observations of non-independent fluxes is that of a layer of sites at the cell surface. The external concentration of a particular ion is supposed to be effective in promoting inward ion movement only to the extent to which the ions occupy sites in a region at the cell surface. It is assumed that the ions in the external solution equilibrate with ions in the surface layer at a rate much higher than that for entrance into the cell from the surface layer so that, to a first approximation, equilibrium can be regarded as established between the external solution and the surface layer. Ions are assumed to proceed into the cell interior from the surface region at a rate proportional to their concentration in the layer. The condition for equilibrium is that the chemical potentials in the external solution and in the surface layer be equal. The ionic activity in the external solution is given by γC in which C is concentration and γ is the activity coefficient. To simplify the treatment, γ is assumed to be constant and equal to unity so that the solution activity is given by C. In the surface layer there are assumed to be N total sites, n_1 of which are

assumed to be occupied by ions of species 1. Fowler (18) has shown on statistical mechanical grounds that the activity in an adsorption layer or surface layer of sites may be represented by $\frac{\theta}{1-\theta}$ in which θ is the fraction of sites occupied. Thus for species 1, $\theta_1 = n_1/N$ and the activity in the layer is given by $a_{ads} = \frac{n_1}{N - n_1}$ in which the subscript ads refers to the adsorbed layer. The chemical potential of an ion in the surface layer is then given by:

$$\mu_{ads} = \mu^{\circ}_{ads} + RT \ln \frac{\theta_1}{1 - \theta_1} \tag{1}$$

That of an ion in the external solution is given by:

$$\mu_{soln} = \mu^{\circ}_{soln} + RT \ln a_{soln} \tag{2}$$

Assuming an activity coefficient of 1 in the solution and equilibration of the solution ions with the ions in the surface phase, one obtains from the relationship $\mu_{soln} = \mu_{ads}$:

$$\frac{\theta_1}{(1 - \theta_1)C_1} = \frac{n_1}{(N - n_1)C_1} = \exp (1/RT)(\mu^{\circ}_{soln} - \mu^{\circ}_{ads})$$

In which C_1 is the concentration in the solution. The quantity $\mu^{\circ}_{soln} - \mu^{\circ}_{ads}$ is seen to be equal to $-\Delta\mu^{\circ}_{ads}$ in which $\Delta\mu^{\circ}_{ads}$ is the standard molal free energy of adsorption. The quantity obviously represents the free energy change when 1 gram mole of ions passes from the standard state in the solution to the standard state in the surface layer. The adsorbed standard state is that of one-half saturation.

Solving the above equation for n_1, one obtains:

$$n_1 = N \frac{k_1 C_1}{k_1 C_1 + 1} \tag{3}$$

where

$$k_1 = e^{-\frac{\Delta\mu^{\circ}_{ads}}{RT}} \tag{4}$$

By setting the uptake rate proportional to the number of occupied sites the following equation is obtained:

$$\phi_1 = P_1 n_1 = P_1 N \frac{k_1 C_1}{k_1 C_1 + 1} \tag{5}$$

The theory applied thus predicts that the uptake of a single ion species in the absence of competitors should follow an equation having the form of equation

(5). The proportionality factor P_1 is assumed to be constant and hence the product P_1N is a constant.

When another species of ion is present also having access to the same sites, the number of sites available to the original ion species will be reduced. To obtain a quantitative relation which holds for two competing ion species one proceeds as before recalling that the activity in the surface layer is given by the ratio of the number of occupied sites to the number of sites available for occupation. Letting the number of unoccupied sites be denoted by α and equating equations (1) and (2) for each ion species 1 and 2, the following equations are obtained:

$$\frac{n_1}{\alpha C_1} = k_1 \quad (6) \quad \text{and} \quad \frac{n_2}{\alpha C_2} = k_2 \qquad (7)$$

Eliminating α from these equations one obtains:

$$n_2 = \frac{k_2 C_2}{k_1 C_1} n_1 \qquad (8)$$

It is obvious that the number of unoccupied sites is given by $\alpha = N - n_1 - n_2$. Equation (6) may therefore be rewritten as follows:

$$\frac{n_1}{N - n_1 - n_2} = k_1 C_1 \qquad (9)$$

Substituting the value for n_2 given in equation (8) and solving for n_1 yields:

$$n_1 = N \frac{k_1 C_1}{k_1 C_1 + k_2 C_2 + 1} \qquad (10)$$

Proceeding as in equation (5), one obtains for the flux of species 1 in the presence of species 2:

$$\phi_{1,2} = P_1 N \frac{k_1 C_1}{k_1 C_1 + k_2 C_2 + 1} \qquad (11)$$

The theory stated predicts that the flux of an ion species should be lowered in the presence of competitor ions of a different species in accordance with equation (11).

It is evident from equation (4) that the constants multiplying each concentration are related to the free energy changes occurring when ions move from the external solution into the surface layer. They are thus measures of the affinities of the ions for the sites. Ions with large affinity constants will be good competitors on this basis.

Equations (5) and (11) can be derived less formally as follows. It is assumed

that entrance from the solution into the surface layer of the membrane requires a collision between an ion in the external solution and a vacant available site in the surface. The loss of ions from the layer back into the external solution, however, is assumed to depend only on the number of ions in the surface and not on the number of sites yet available for occupation. Let the rate at which ions enter the surface from the solution be given by j_1 and the rate at which they pass from the surface back into the external solution be given by j_1'.

From the above discussion,

$$j_1 = a\, C_1(N - n_1) \tag{12}$$

and

$$j_1' = b\, n_1 \tag{13}$$

in which a and b are constants. When the surface layer and the external solution ions are in equilibrium, equations (12) and (13) can be equated. Setting $j_1 = j_1'$ and solving the resulting equation for n_1 one obtains:

$$n_1 = N \frac{(a/b)C_1}{(a/b)C_1 + 1} \tag{14}$$

Equation (14) is identical with equation (3) with $a/b = k_1$. Equation (5) is easily obtained from equation (14). To obtain equation (11) by this method one equates equations (12) and (13) for ions of species 1 and for ions of species 2 in the same mixture so that the number of yet available sites α will be the same for both species. If α is eliminated from the resulting equations, an equation similar to equation (8) is obtained. One then proceeds through the same steps used in equations (9) and (10) to derive equation (11). In the latter analysis, the constant multiplying each concentration is the ratio of two rate constants. Such a ratio is often called an equilibrium constant. Thermodynamically, the equilibrium constant is related to a standard molal free energy change by a relationship similar to equation (4). The two derivations are, therefore, consistent.

The reasoning used to derive the foregoing equations is formally identical with that used in deriving the so called Langmuir adsorption isotherm. The equations thus have a formal resemblance to such isotherms. It is also possible to derive equations like (5) and (11) by various different but closely related means. One such means is to regard ions as reacting with a carrier molecule of some sort which then transports them to the inner cell membrane boundary where they are unloaded and diffuse into the aqueous phase. Such models are based on similar bimolecular reaction kinetics. One such derivation is presented by Epstein (13).

Experimental To see whether the model presented can account for some of the non-independent fluxes observed, one can proceed along several lines experimentally. One procedure would be to see whether the fluxes of single species of ions follow an equation having the form of equation (5). If such is the case, one could evaluate the affinity constant for each of the three ions K, Rb, and Cs from single ion species experiments with no other competing species present. A rigorous test of the model would be to see whether or not the same affinity constants correctly predict the effect of the presence of ions of a different species on the fluxes of the three ions. This was the procedure adopted in the first part of this work. It was found that the uptakes of the three ions K, Rb, and Cs could be fitted to three equations each having the form of equation (5). The data for single ion movements are presented in Table II. In each case the observed fluxes at various concentrations are compared with values calculated from an equation having the same form as equation (5). The constants required in each equation are given in the table. The constants in these equations analogous to PN in the derived equations obviously have the dimensions of flux. In the formal treatment, PN is evidently the maximum flux attainable when all N of the sites become occupied by the ion species traced. Experimentally these constants are, of course, those required to fit the data. According to the theory developed, the flux of each ion should approach the value of the respective constant PN at very high external concentrations. With cesium ions the agreement at a high concentration (100 mM) is very good. Cesium ions do not appear to be near saturation of the sites at a concentration of 100 mM, however, and it is not known whether agreement would be obtained at still higher concentrations since 100 mM is the highest concentration used in these experiments. For rubidium ions the calculated value at a concentration of 100 mM falls below the measured value, but the divergence is not great. For potassium ions the calculated value of the flux at a concentration of 100 mM falls considerably below the measured value. These deviations are not considered to be serious since agreement for all three ions is excellent over a wide range in concentration. In addition, the tendency for disagreement at high concentrations may have an adequate explanation. There may be alternate pathways for ion penetration which only become a significant fraction of the total inward movement at very high concentrations. One such pathway may be a cation-anion paired movement. It is known, for example, that considerable KCl entrance takes place at high KCl concentrations in frog muscle. When the additional KCl is substituted for NaCl in the medium, the KCl entrance is accompanied by swelling and the net water movement maintains the intracellular potassium concentration near the normal value (Boyle and Conway (19)). When the additional KCl is added to the normal NaCl of the medium, an initial shrinkage occurs according to Boyle and Conway followed by a prolonged maintaining of the normal weight.

TABLE II

UPTAKE RATES OF POTASSIUM, RUBIDIUM, AND CESIUM IONS COMPARED WITH VALUES CALCULATED FROM THEORY

A. Potassium ions $\phi_K = 133 \frac{23.2\, C_K}{23.2\, C_K + 1}$ *

C_K	ϕ_K observed	ϕ_K calculated
mM	*μmoles/gm. hr.*	
2	5.9	5.9
2.5	7.8	7.3
4	11.3	11.3
5	13.9	13.9
8	19.7	20.9
10	22.0	25.1
15	30.0	34.2
20	39.1	42.3
40	64.0	64.1
100	138	93.0
100 (sulfate medium)‡	88.0	93.0

B. Rubidium ions $\phi_{Rb} = 20 \frac{83.2\, C_{Rb}}{83.2\, C_{Rb} + 1}$

C_{Rb}	ϕ_{Rb} observed	ϕ_{Rb} calculated
mM	*μmoles/gm. hr.*	
0.2	0.325	0.331
0.6	0.877	0.952
2	3.1	2.9
2.5	3.6	3.5
5	5.1	5.9
10	8.0	9.1
20	12.8	12.5
40	13.5	15.4
100	20.0	17.9

C. Cesium ions $\phi_{Cs} = 34 \frac{14.0\, C_{Cs}}{14.0\, C_{Cs} + 1}$

C_{Cs}	ϕ_{Cs} observed	ϕ_{Cs} calculated
mM	*μmoles/gm. hr.*	
0.6	0.23	0.28
2.5	1.1	1.2
5	2.5	2.2
10	4.6	4.2
12	4.3	4.9
20	7.0	7.4
40	11.6	12.2
60	15.6	15.5
100	19.6	19.8

* For numerical convenience, concentrations in equations are expressed in units of moles per liter while concentrations in tables are expressed as millimoles per liter.

‡ Composition of sulfate solution used: $NaHCO_3$ 30 mM, K_2SO_4 50 mM, $Ca(NO_3)_2$ 2 mM, sucrose 40 mM, 95 per cent O_2–5 per cent CO_2 gas mixture.

In the latter case, a net gain of KCl occurs at constant weight so that the intracellular potassium concentration rises markedly. In both cases the additional KCl uptakes are of comparable magnitude. A test of the plausibility of this explanation would be the measurement of potassium uptake at a concentration of 100 mM with a non-penetrating anion replacing the chloride in the medium. The non-penetrating anion selected was sulfate ion. The measured

TABLE III

UPTAKE RATES OF POTASSIUM IONS FROM BI-IONIC MIXTURES COMPARED WITH VALUES CALCULATED FROM THEORY

A. Potassium ions in the presence of rubidium ions

$$\phi_K = 133 \frac{23.2\, C_K}{23.2\, C_K + 83.2\, C_{Rb} + 1}$$

C_K	C_{Rb}	ϕ_K observed	ϕ_K calculated
mM	mM	μmoles/gm. hr.	
2.5	0	7.8	7.3
2.5	2.0	5.6	6.3
2.5	10	3.7	4.1
2.5	15	3.6	3.3
2.5	20	2.6	2.8
2.5	25	2.3	2.5
2.0	40	1.2	1.4
2.0	60	0.90	1.0
2.0	100	0.66	0.66

B. Potassium ions in the presence of cesium ions

$$\phi_K = 133 \frac{23.2\, C_K}{23.2\, C_K + 14.0\, C_{Cs} + 1}$$

C_K	C_{Cs}	ϕ_K observed	ϕ_K calculated
mM	mM	μmoles/gm. hr.	
2.5	0	7.8	7.3
2.5	20	4.6	5.8
2.5	30	4.7	5.2
2.5	40	3.8	4.8

flux of potassium ions was lowered to 88 μ M/gm. hr. with no chloride ions present in the medium. This value is in good agreement with the calculated value of 93 μ M/gm. hr. indicating that the above explanation is quantitatively a possible one. Little swelling occurs in high concentrations of CsCl indicating a very low rate of CsCl entrance. This is consistent with the fact that no great deviations were found when high concentrations of Cs ions were employed.

The next phase of the investigation was conducted to see whether interspecies competition could be described by equations of the form of equation (11) using the same affinity constants evaluated from the single ion species ex-

periments. It is to be emphasized that no new constants are to be evaluated in making this test nor are any changes in constants already evaluated permitted. The results are presented in Tables III to V. The measured fluxes of each of the ion species in the presence of various concentrations of competitor ions of a different species are compared with values calculated from equations having the same form as equation (11) and containing appropriate constants. In every case the equation used is given in the table.

TABLE IV

UPTAKE RATES OF RUBIDIUM IONS FROM BI-IONIC MIXTURES COMPARED WITH VALUES CALCULATED FROM THEORY

A. Rubidium ions in the presence of potassium ions

$$\phi_{Rb} = 20 \frac{83.2\, C_{Rb}}{83.2\, C_{Rb} + 23.2\, C_K + 1}$$

C_{Rb}	C_K	ϕ_{Rb} observed	ϕ_{Rb} calculated
mM	*mM*	*μmoles/gm. hr.*	
2.5	0	3.6	3.5
2.5	5	2.8	3.1
2.5	10	2.7	2.9
2.5	15	2.4	2.7
2.0	40	1.3	1.6
2.0	100	0.81	0.96

B. Rubidium ions in the presence of cesium ions

$$\phi_{Rb} = 20 \frac{83.2\, C_{Rb}}{83.2\, C_{Rb} + 14.0\, C_{Cs} + 1}$$

C_{Rb}	C_{Cs}	ϕ_{Rb} observed	ϕ_{Rb} calculated
mM	*mM*	*μmoles/gm. hr.*	
2.5	0	3.6	3.5
2.5	5	3.0	3.2
2.5	10	2.8	3.1
2.5	20	2.7	2.8
2.5	40	2.1	2.3

From the constants evaluated in single ion species experiments, one can easily make qualitative predictions of the extent to which the ions should compete in various bi-ionic mixtures. The affinity constant for rubidium ions appears to be about 3.6 times greater than that for potassium ions and 6 times greater than that for cesium ions. The flux of cesium ions should, if theory holds, be greatly lowered from the control value in the presence of rubidium ions. Rubidium flux, on the other hand, should be only modestly lowered in the presence of cesium ions. These expectations are borne out by the data presented in the tables. If flux values in the presence of 20 mM of the competitor are compared with control values in the absence of competitor, for example,

it is found that rubidium ions have lowered the cesium flux to 28 per cent of the control value. Cesium ions at the same concentration, on the other hand, have only lowered the rubidium flux to 75 per cent of the control value. Other values given in the tables seem to support the conclusion that the theory applied is basically a valid one, at least as a first approximation.

The experimental procedure thus far has shown that single ion species movements contain sufficient information to predict the extent of competition

TABLE V

UPTAKE RATES OF CESIUM IONS FROM BI-IONIC MIXTURES COMPARED WITH VALUES CALCULATED FROM THEORY

A. Cesium ions in the presence of potassium ions

$$\phi_{Cs} = 34 \frac{14.0\, C_{Cs}}{14.0\, C_{Cs} + 23.2\, C_K + 1}$$

C_{Cs}	C_K	ϕ_{Cs} observed	ϕ_{Cs} calculated
mM	*mM*	*μmoles/gm. hr.*	
10	0	4.6	4.2
10	10	3.3	3.5
10	20	2.7	3.0
10	40	1.7	2.3
10	80	1.7	1.6

B. Cesium ions in the presence of rubidium ions

$$\phi_{Cs} = 34 \frac{14.0\, C_{Cs}}{14.0\, C_{Cs} + 83.2\, C_{Rb} + 1}$$

C_{Cs}	C_{Rb}	ϕ_{Cs} observed	ϕ_{Cs} calculated
mM	*mM*	*μmoles/gm. hr.*	
10	0	4.6	4.2
10	10	1.8	2.4
10	20	1.3	1.7
10	40	1.3	1.1
10	80	0.82	0.61

offered by ions in bi-ionic mixtures. One could have proceeded conversely by attempting to show that ion movements observed in mixtures provide sufficient information to describe the individual ion movements separately. This was not done in the foregoing experiments because the single ion fluxes are known most accurately for reasons presented. If a means of plotting were available that laid greater emphasis on the later uptake points, it is possible that information provided in mixture experiments would be made as accurate as that coming from single ion experiments. One such technique of plotting is to plot uptake *versus* the square root of the time. Harris (20) has shown that potassium uptakes in frog muscle show a large interval in which uptake is a

linear function of the square root of time. He pointed out that this result would be expected if diffusion played a prominent role in potassium movements in muscle. Potassium uptake from the present experiments is plotted against the square root of time in Fig. 3. Also included is a series of plots of potassium

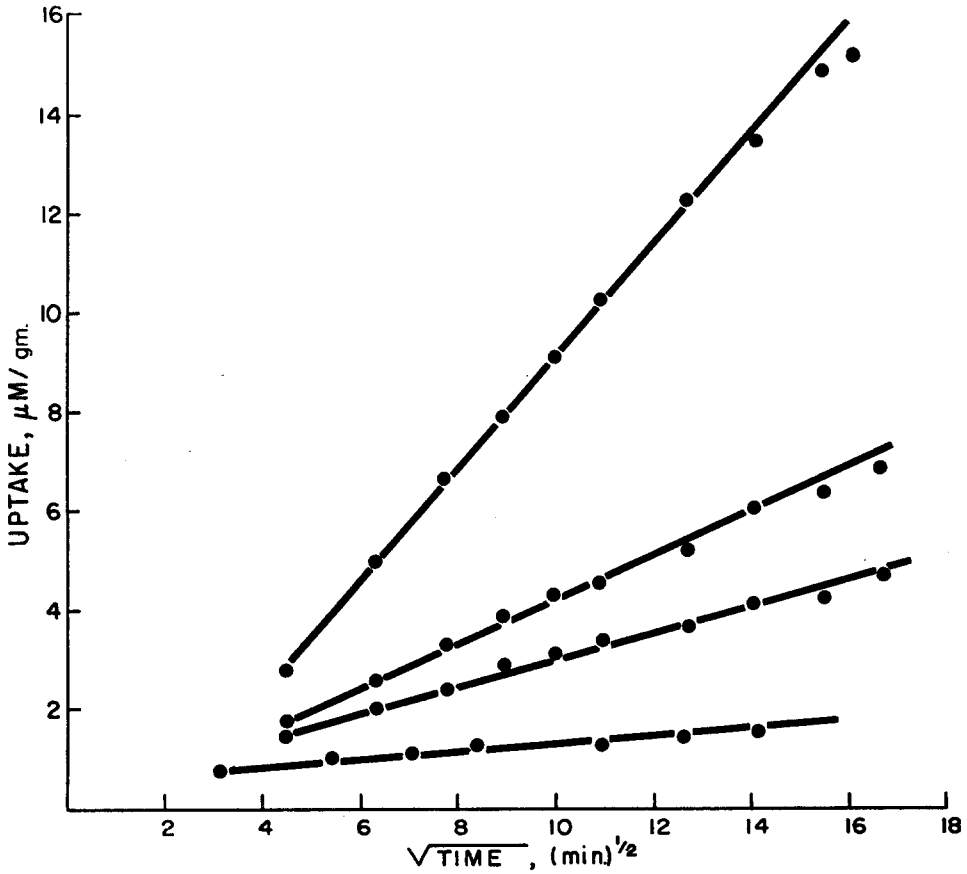

FIGURE 3. Experimentally determined potassium uptakes are plotted against the square root of time. For all curves the external potassium ion concentration is 2.5 mM. The rubidium contents of the solutions are, from top to bottom, (1) no rubidium, (2) 15 mM, (3) 25 mM, (4) 60 mM.

uptake in the presence of various concentrations of rubidium ions. The slopes of the straight lines obtained are expressed in units of μM/gm.(min.)$^{\frac{1}{2}}$. The slopes obtained are not fluxes nor are they convertible to flux by any derivable mathematical procedure. A single exponential equation of the form previously stated, however, is seen from Fig. 4 to have a considerable linear portion when replotted against the square root of time. Though the square root slopes are not necessarily numerically proportional to the fluxes, they are obviously a

numerical measure of the rapidity of exchange. If two uptakes are considered and one is found to have a larger square root slope, the finding is a direct consequence of the case with larger slope also having a larger initial flux. If all results are plotted in this way and the best straight line is drawn through the points over a comparable interval of time in each case, consistent and

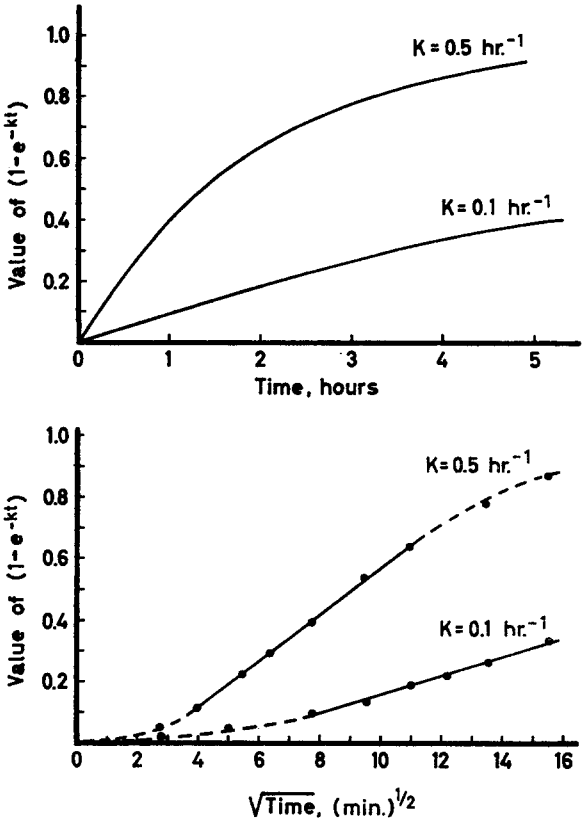

FIGURE 4. In the upper graph, the function $(1 - e^{-kt})$ is plotted for two values of the rate constant (upper curve, $k = 0.5$ hr.$^{-1}$; lower curve, $k = 0.1$ hr.$^{-1}$). In the bottom graph, the points are various values of the same function replotted against the square root of time.

comparable numerical estimates of the rapidity of exchange should be obtained. In comparing this method with the method of obtaining fluxes, it can be stated that the square root plots lay greater emphasis on later uptake points in the cases of movements from mixtures. The method of obtaining fluxes requires the subtraction of an estimate of the ions residing in the extracellular space while the square root method requires no such subtraction since uptake is linear after a delay in which the extracellular space has had time to equilibrate.

Tables VI to VIII contain the experimental data obtained reported as square root of time slopes. As before, each observed value is compared with a value calculated from equations given. In this treatment, however, the affinity

TABLE VI

OBSERVED POTASSIUM UPTAKE SQUARE ROOT OF TIME SLOPES COMPARED WITH CALCULATED VALUES

All slopes are in units of μmoles K/gm. (min.)$^{1/2}$

A. K ions with no competitors

$$S_K = 8.0 \frac{71.5\, C_K}{71.5\, C_K + 1}$$

C_K	S_K observed	S_K calculated
mM		
2.0	1.0	1.1
2.5	1.2	1.2
5.0	2.2	2.1
10.0	3.6	3.3
15.0	4.1	4.1
20.0	5.2	4.7
40.0	5.8	5.9
100.0	11.8	7.0
100.0 (sulfate medium)*	7.9	7.0

B. K ions in presence of Rb ions

$$S_K = 8.0 \frac{71.5\, C_K}{71.5\, C_K + 143\, C_{Rb} + 1}$$

C_K	C_{Rb}	S_K observed	S_K calculated
mM	*mM*		
2.5	2	0.90	0.98
2.5	15	0.44	0.43
2.5	20	0.34	0.35
2.5	25	0.28	0.30
2.0	40	0.16	0.17
2.0	60	0.12	0.12
2.0	100	0.084	0.074

C. K ions in presence of Cs ions

$$S_K = 8.0 \frac{71.5\, C_K}{71.5\, C_K + 53.6\, C_{Cs} + 1}$$

C_K	C_{Cs}	S_K observed	S_K calculated
mM	*mM*		
2.5	20	0.60	0.63
2.5	30	0.55	0.51
2.5	40	0.40	0.43

* Composition of sulfate solution used: $NaHCO_3$ 30 mM, K_2SO_4 50 mM, $Ca(NO_3)_2$ 2 mM, sucrose 40 mM, 95 per cent O_2–5 per cent CO_2 gas mixture.

constants were chosen to provide the best fit in the mixture cases. The results obtained show a consistency with the results obtained from the data reported as flux. The same order is observed for the affinity constants of the three ions. The *PN* constants are also comparable but all constants have different numerical proportions which would be expected for an entirely different method of plotting.

TABLE VII

OBSERVED RUBIDIUM UPTAKE SQUARE ROOT OF TIME SLOPES COMPARED WITH CALCULATED VALUES

All slopes are in units of μmoles Rb/gm. (min.)$^{1/2}$

A. Rb ions with no competitors

$$S_{Rb} = 2.6 \frac{143\, C_{Rb}}{143\, C_{Rb} + 1}$$

C_{Rb}	S_{Rb} observed	S_{Rb} calculated
mM		
2.0	0.64	0.58
2.5	0.70	0.68
5.0	1.03	1.08
10.0	1.50	1.53
20.0	1.71	1.92
40.0	1.90	2.21
100.0	3.42	2.44

B. Rb ions in presence of K ions

$$S_{Rb} = 2.6 \frac{143\, C_{Rb}}{143\, C_{Rb} + 71.5\, C_K + 1}$$

C_{Rb}	C_K	S_{Rb} observed	S_{Rb} calculated
mM	*mM*		
2.5	5	0.52	0.54
2.5	10	0.44	0.45
2.5	15	0.35	0.38
2.0	40	0.19	0.18
2.0	100	0.086	0.088

C. Rb ions in presence of Cs ions

$$S_{Rb} = 2.6 \frac{143\, C_{Rb}}{143\, C_{Rb} + 53.6\, C_{Cs} + 1}$$

C_{Rb}	C_{Cs}	S_{Rb} observed	S_{Rb} calculated
mM	*mM*		
2.5	5	0.51	0.57
2.5	10	0.45	0.49
2.5	20	0.40	0.38
2.5	40	0.27	0.27

The single ion agreement by this method is good until concentrations above 40 mM are reached. Above this concentration, the square root slopes observed lie considerably above the calculated values. The reason for this may be the same one previously discussed, namely, that paired cation-anion movement provides an alternate pathway for uptake which becomes a significant fraction of the total uptake only at very high concentrations. The square root slope for potassium ions at a concentration of 100 mM in a sulfate medium (presented

TABLE VIII

OBSERVED CESIUM UPTAKE SQUARE ROOT OF TIME SLOPES COMPARED WITH CALCULATED VALUES

All slopes are in units of μmoles Cs/gm. (min.)$^{1/2}$

A. Cs ions with no competitors

$$S_{Cs} = 2.6 \frac{53.6\, C_{Cs}}{53.6\, C_{Cs} + 1}$$

C_{Cs}	S_{Cs} observed	S_{Cs} calculated
mM		
2.5	0.28	0.31
5.0	0.60	0.55
10.0	0.89	0.91
20.0	1.39	1.34
40.0	1.65	1.77
60.0	2.10	1.98
100.0	3.05	2.20

B. Cs ions in presence of K ions

$$S_{Cs} = 2.6 \frac{53.6\, C_{Cs}}{53.6\, C_{Cs} + 71.5\, C_K + 1}$$

C_{Cs}	C_K	S_{Cs} observed	S_{Cs} calculated
mM	mM		
10	10	0.57	0.62
10	20	0.47	0.47
10	40	0.30	0.32
10	80	0.22	0.19

C. Cs ions in presence of Rb ions

$$S_{Cs} = 2.6 \frac{53.6\, C_{Cs}}{53.6\, C_{Cs} + 143\, C_{Rb} + 1}$$

C_{Cs}	C_{Rb}	S_{Cs} observed	S_{Cs} calculated
mM	mM		
10	10	0.51	0.47
10	20	0.27	0.32
10	40	0.15	0.19
10	80	0.085	0.11

in table) is much nearer the calculated value. Another means to estimate the amount of cation uptake that is paired with chloride ions is to use the time course of swelling at high concentrations of KCl, RbCl, and CsCl when substituted for NaCl in the medium as an index of the time course of net gain of salt. As was previously mentioned, the amount of net gain seemed to be comparable in magnitude for KCl whether or not the cells swelled to main-

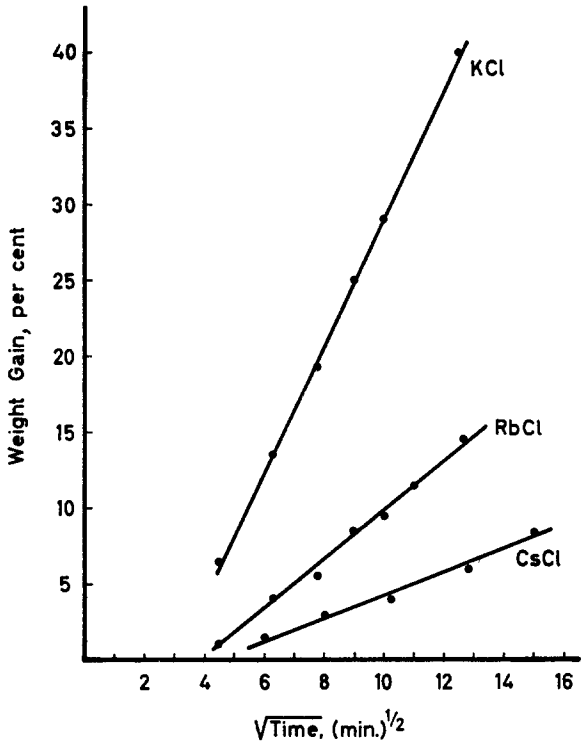

FIGURE 5. The weight gains observed when muscles were placed in solutions in which the normal NaCl was replaced with 100 mM of KCl, RbCl, or CsCl are plotted against the square root of time. The solution compositions were: $NaHCO_3$ 20 mM, $CaCl_2$ 2 mM, plus 100 mM of KCl or RbCl or CsCl. The solutions were bubbled with a 95 per cent O_2–5 per cent CO_2 gas mixture.

tain a constant potassium concentration. The percentage increase in weight of muscles in solutions with 100 mM of KCl, RbCl, and CsCl respectively replacing most of the NaCl of the medium is plotted against $\sqrt{t}$ in Fig. 5. One member of the pair of muscles used for each net gain estimation was placed in the same solution as its mate was in for an initial period (about 10 minutes) until the weight gain became linearly related to $\sqrt{t}$. At this time one muscle was removed for cation analysis by flame photometry. At the end of the experiment the other muscle was analyzed for cations. Both members of the pair

were assumed to have the same extracellular content of cations since both were in the same solution for an initial period in which extracellular equilibration was essentially complete. The net gain was therefore estimated by subtraction. In the case of KCl movement, the difference in potassium content of the two muscles of the pair occurred over a known interval of time in which it was also known that the net gain was linear with $\sqrt{t}$. The square root slope of the net gain or KCl entrance could then be obtained by dividing the potassium difference obtained by analysis by the initial weight and by the square root of the time interval. As an example using experimental data, one muscle weighed 60 mg., its mate weighing 61 mg. initially. The 60 mg. muscle was removed from the solution after 10 minutes and was found to contain 5.61 μmoles of potassium. The other muscle was removed from the solution 150 minutes later and was found to contain 8.55 μmoles of potassium. The difference of 2.94 μmoles then represents the net gain of potassium which occurred in the interval. The number of square root units which occur between 10 minutes and 150 minutes is 9.0 $(\text{min.})^{\frac{1}{2}}$. The net gain square root slope is then 2.94 μmoles divided by 9.0 $(\text{min.})^{\frac{1}{2}} \times 0.060$ gm. The resulting slope is 5.4 $\mu\text{M/gm.}(\text{min.})^{\frac{1}{2}}$. The observed slope from tracer movement in 100 mM KCl was 11.8 $\mu\text{M/gm.}(\text{min.})^{\frac{1}{2}}$. The difference between the two is 6.4 which represents the exchange slope and agrees fairly well with the calculated value of 7.0. In another experiment the net gain slope determined was 3.8. This amount subtracted from 11.8 leads to a slope of 8.0. Better agreement is thus obtained if an estimate of the anion-paired movement is subtracted at very high concentrations. The same procedure can be carried out for RbCl and CsCl with the exception that analyses must be carried out for potassium as well as for the other ion present. Since potassium is being lost from the cells in these cases, net gains of total cation can only be due to either the RbCl or the CsCl present. Slopes obtained for RbCl and for CsCl were 1.1 and 0.61 respectively. Subtraction of these values from the slopes found from tracer uptakes leads to values of 2.3 and 2.4 respectively for Rb and Cs at a concentration of 100 mM. These values compare favorably with the calculated slopes.

The slopes obtained from net salt gains are obtained with a method somewhat cruder than the tracer technique and are included only to show that the disagreement observed at 100 mM concentrations can plausibly be accounted for by such an explanation. It is to be noted that the treatment in this work does not depend critically on the correctness of the net gain explanation for a very small fraction of the experimental values shows deviations of this kind. From concentrations of 40 mM and below, agreement is quantitative to within experimental error.

A Note on Experimental Errors Due to the rather large number of experimental points necessary to test the competition equations, it was not possible

to repeat experiments on each point. In the course of the experiments, many duplications were required because of the necessity for controls. Experiments for which multiple points are available are reported in Table IX. In each group, the standard error is determined and reported as a percentage of the mean. Finally, an average standard error is determined by weighting each

TABLE IX

RESULTS USED TO ESTIMATE EXPERIMENTAL ERROR

Ion traced	Concentration (mM) of ions competing with tracer flux		Uptake slopes	Average ± standard deviation
			μmoles/gm. (min.)$^{1/2}$	
K	K	2.5	1.17	
			1.40	1.21±0.12
			1.10	
			1.24	100±9.7
			1.15	
K	K	10.0	3.35	3.55±0.28
			3.74	100±8.0
K	K	100	11.6	11.8±0.16
			11.9	
			11.8	100±1.35
K	K	2.0	0.075	0.084±0.013
	Rb	100	0.093	100±15.5
Rb	Rb	2.0	0.68	
			0.65	0.67±0.025
			0.70	100±3.7
			0.65	
Rb	Rb	100	3.25	
			3.34	3.42±0.20
			3.70	100±5.8
			3.40	
Rb	Rb	2.0	0.092	0.086±0.0085
	K	100	0.080	100±9.9
Cs	Cs	10.0	0.88	0.89±0.022
			0.91	100±2.5
Cs	Cs	20.0	1.39	1.33±0.085
			1.26	100±6.4
Cs	Cs	100	3.00	3.05±0.07
			3.10	100±2.3
Average standard deviation weighted according to the No. of observations in each group (total observations = 28):				100±6.5

individual group standard error according to the number of observations in the group. Out of a total of 28 observations, the average standard error was found to be about ± 7 per cent. Though the data are limited, it is hoped that this procedure provides a reasonable estimate of the average experimental error. With this distribution of error, experimental results which differ by 10 to 15 per cent will be rather frequent. Results which differ by over 20 per cent will tend to be relatively infrequent while results differing by 28 per cent or over will be rare.

A test of the theoretical points would obviously be to regard them as mean values and to see whether or not the experimental points appear to be randomly distributed about the mean values with a standard error of about ± 7 per cent. If this test is applied to the square root slopes presented, it is found that out of a total of 48 test points, 59 per cent of the observations lie within ± 1 standard deviation of the theoretical values, 92 per cent lie within ± 2 standard deviations, and 100 per cent lie within ± 3 standard deviations. The agreement of the data with theory would seem to be within experimental error.

DISCUSSION

It appears satisfactory to describe cation competition in frog sartorius muscle in terms of the theoretical equations presented. One might then inquire further into the possible significance of these equations. One possibility is that the equations are of the correct form and contain sufficient constants to fit the data by merely applying the techniques of curve fitting. An argument against this notion is that nine different combinations of competition between nontracer and tracer movements are fitted satisfactorily over a wide concentration range without alteration of constants. A further argument is that the affinities or selectivities found lie in an order which, though different from the order of rate, is the same as the order in which muscle cells selectively concentrate the ions after equilibration in various mixtures. Lubin and Schneider (21) for example, find that sartorius muscle cells selectively concentrate the three ions in the order Rb > K > Cs, the same order observed for the affinity constants in the present work. Both types of experiments, taken together, support the notion that the model applied is basically a sound one.

It is difficult on the basis of the evidence presented to advance any ideas as to the physical-chemical nature of the postulated sites. Though typical cation exchange membranes show selectivity to a series of cations, the degree of selectivity exhibited is usually much less than noted for muscle cells in this work. In addition, the order of selectivity is different for the majority of cation exchangers, cesium ions being preferred slightly more than rubidium ions which are in turn preferred slightly more than potassium ions. Typical anionic

charged groups do not seem to be likely candidates for the sites postulated at the muscle cell surface. Clearly, information is lacking on the detailed structure of cell membranes as well as on the relative roles of membrane and non-membrane parts of the cell in setting limits on ion exchange rates in muscle. At present, it appears best to restrict the discussion to a few generalizations which are evident. Table X compares the biological differences noted for the three ions with a few of their physical parameters. The first feature that one notices from the table is the extremely large biological differences in contrast to the rather small physical differences. The most obvious correlation that one observes in the table is that which exists between the penetration rate and the crystal radii of the ions. The numbers appearing in the table are not inconsistent with the notion that ions are screened at the barrier to penetration on the basis of their crystal radii, ions with smaller radii showing superior penetration rates. Mullins (5, 22) has discussed the possible physical basis for

TABLE X

SOME PHYSICAL PROPERTIES OF K, Rb, AND Cs IONS COMPARED WITH SOME OF THEIR PARAMETERS IN MUSCLE

Ion	Crystal radius	Hydrated radius	Equivalent conductance (aqueous)	Apparent muscle relative affinity		Penetration rate 2.5 mM
				From square root slopes	From fluxes	
	Å	Å				μM/gm. hr.
K	1.33	2.52	64.4	1.0	1.0	7.8
Rb	1.48	2.37	67.5	2.0	3.6	3.6
Cs	1.69	2.19	67.7	0.75	0.60	1.1

this correlation in detail. One must reconcile this correlation, however, with the finding that the apparent affinities do not seem to lie in an order readily correlatable with ion size. Ling (8) for example, suggests that cation specificity in muscle depends on the hydrated radii of the ions which are supposed to limit their closeness of approach to fixed anionic sites within the cell. Table X shows that the potassium and rubidium order is not inconsistent with this hypothesis but that the position of the cesium ion is entirely wrong for agreement. Cesium ions, according to Ling's hypothesis, should show the properties of potassium ions in exaggeration. Actually, they tend to behave more like sodium ions in muscle with regard to both affinity and penetration rate. The effect of cesium on the muscle membrane potential also fits this notion (Sjodin (1)). The situation seems to be more complicated than any simple scheme would indicate. The data bring out strongly the twofold nature of the penetration or exchange process, namely the dependence of the over-all rate on (*a*) a partitioning of the ions between the external solution and the membrane, and (*b*) the mobility of each ion within the permeability barrier. Com-

petition studies may provide a means for distinguishing the two effects. Rubidium ions may be more powerful competitors because they are partitioned to the membrane in greater number. A pore size distribution, for example, may be "peaked" near the rubidium crystal radius size (Mullins (5)). This could account for both potassium and cesium showing lower affinities than rubidium. One would then have to postulate a much lower membrane mobility for Rb ions than for K ions. Another possibility is that the affinities and the mobilities are not independent. The slower ion may, for example, tend to accumulate in the membrane and thus show an apparently larger affinity. This type of mechanism could account nicely for the K and Rb data since the Rb ion is slower than the K ion by about a factor of two and is twice as good a competitor. This reasoning fails completely, however, with cesium in which case the slowest ion is seen to be the weakest competitor. Here one would have to invoke the notion of a definite partition specificity as the limiting factor. That is, relatively few cesium ions are expected to be found in the surface layer of the membrane at a given applied concentration.

A question which arises is the relation of the specificity to cations discussed here to that already observed in muscle with regard to sodium and potassium ions. This was checked very briefly by noting the effect of Rb ions on sodium output as compared with that of K ions. An enchancement of sodium efflux has been observed in muscle in the presence of K ions over that observed in their absence (Harris (23), Keynes (24)). This suggests the operation of a coupled K and Na exchange process. It was of interest to learn the ability of Rb ions to participate in this exchange process. The finding was that rubidium ions had to be used at twice the concentration of potassium ions to obtain the same enhancement of sodium efflux. Table XI shows that the percentage increase in rate constant for Na efflux over that observed in a K- and Rb-free solution is about twice as high for K ions when expressed per unit of applied concentration. The result suggests that K and Rb ions participate in sodium exchange in proportion to their penetration rates and not to their affinities. The investigation of this point is far from complete but suggests that the K, Rb, and Cs specificity lies at a different level than the sodium specificity. It could be argued, for example, that the bulk of the experimental results presented can be explained by assuming that all potassium entry is through a soidium-coupled "pump." The lower entry rates for potassium in the presence of rubidium and cesium ions would then be explained by assuming that the latter ions poison the pump to different degrees. This explanation does not seem adequate as the ion which would have the greater poisoning capacity, rubidium, actually enters at a rate superior to cesium ion which would, supposedly, be the lesser poison. Another fact that argues against the notion that a sodium-coupled pump is involved to an appreciable extent is that neither sodium nor lithium ions were observed to compete for the postulated sites.

One thus has to assume that sodium and lithium ions have no access to the potassium exchange sites or else have an undetectably low affinity for them. It is possible, then, that potassium exchange sites at the cell surface have little to do with the net movement of potassium ions brought about by coupled anion movement or by coupled exchange with sodium ions.

There does not appear to be any great difference between the type of ion competition described here for muscle cells and that observed by Epstein (13) in barley roots. That the same general picture is observed in two such different samples of living material seems suggestive of similar basic processes underly-

TABLE XI

THE EFFECT OF EXTERNAL RUBIDIUM IONS ON SODIUM EFFLUX COMPARED WITH THE EFFECT OF POTASSIUM IONS

Rate constants for decline* of Na^{24} efflux ($hr.^{-1}$)		K or Rb concentration when present		Ratio	Ratio
				Column 2 / Column 1	Column 2 / Column 1 per unit of applied concentration
1. K andRb absent	2. K or Rb present	K	Rb		
0.51	1.13	—	20 mM	2.21	0.11
0.62	1.35	10 mM	—	2.18	0.22
0.62	1.75	—	100 mM	2.82	0.028
0.57	2.68	100 mM	—	4.70	0.047

* Each muscle was used as its own control in these experiments. The rate constant in the absence of K and Rb ions was first determined for each muscle. The same muscles were then passed into solutions containing K or Rb as specified and the rate constants were again determined. The upper and lower pairs of results were obtained on muscles paired from the same animal. Each pair was used in the same concentration range and so provides directly comparable results. When comparing the lower pair of results with the upper pair, a decreased effectiveness for eliciting increased Na efflux is noted for both ions as the concentration is raised. This may be attributed to the saturation effects discussed.

ing ion movements in perhaps most living cells. Whether or not the various interpretations of such saturation studies are correct, it seems clear that sites or pores which have limited capacity for ions exist in a wide variety of cells. The necessarily resulting saturation tendency may be expected to have profound influences on the behavior of ions when subjected to various driving forces.

It is not suggested that the model used here will describe the movements of the three ion species used under all conditions. The possibility of a paired cation-anion movement has already been mentioned. Also, it is clear that the model developed does not contain the means to handle cases in which the membrane potential has been altered to a new value by the passage of an electric current. In such a case, the ions would be subjected to a definite elec-

trical component of driving force and a different sort of treatment would be required. A formalism often applied to biophysical cases in which the potential difference across the membrane is believed to manifest itself as a driving force is the constant field theory. It is interesting to conjecture how the model used in this work might affect the application of such a theory.

Some Remarks on the Validity of the Constant Field Equation and Boundary Conditions Applied to It The classical notion of an ion moving along a gradient of electrochemical potential has often been applied to ion movements across biological membranes in the form of various constant field equations, especially one derived by Hodgkin and Katz (3) using the constant field theory of Goldman (2). It should be remembered that such equations were derived with the tacit assumption that saturation does not take place and that the membrane is homogeneous throughout. For example, the equation derived by Hodgkin and Katz makes use of a permeability coefficient $P = (RT/F\partial)U\beta$ in which R, T, and F have their usual significance, and in which ∂ is the membrane thickness, U is the membrane mobility, and β is the partition coefficient so that the membrane concentrations of ions at each boundary are given by β times the bulk solution concentrations. This boundary condition neglects the possibility of fixed membrane charges. A constant field equation derived by Teorell (25) takes into account a possible electrical component to the membrane activity by assuming a "Donnan" distribution at the membrane surfaces. For a neutral membrane this boundary condition reduces to that assumed by Hodgkin and Katz.

The assumption of a uniform membrane mobility U may be a permissible and useful first approximation. The assumption of a uniform partition coefficient β, on the other hand, may be a serious error. The assumption of a constant partition coefficient rests on the assumption of a constant activity coefficient within the membrane. The latter assumption rests, in turn, upon the existence of a linear relationship between membrane concentration and membrane activity. If the membrane is assumed to have N fixed sites which an ion can occupy and n of these sites are occupied by a particular ion species, then it is clear that the membrane concentration of that ion species is proportional to n/N by a factor which can be called the "site density." A constant membrane activity coefficient, and hence a constant partition coefficient β, now implies that the membrane activity is proportional to n/N. The electrolyte solution analog of this assumption is well known in the form of the laws of dilute solutions. When n becomes of the order of N in magnitude, however, the activity can be shown to be given by $n/(N - n)$ rather than by n/N as indicated in the thermodynamic derivation of the saturation or adsorption equation given. The membrane activity is approximated by n/N only when n is small compared to N; that is, when the membrane is far from saturation. The many studies mentioned previously all indicate that the membrane is not very far from saturation at physiological concentrations. The constant β assumption is, therefore, not a very valid one. The above expression for the membrane activity, $n/(N - n)$, which can be derived rigorously on statistical mechanical grounds, leads directly to an equation giving β in terms of the external concentration. A formally identical equation was previously derived phenomenologically by Sjodin (1).

The difficulties inherent in the equation derived by Hodgkin and Katz, however, involve more than the need for a more realistic boundary condition. Since the occurrence of saturation in a phase is thermodynamically equivalent to a dependence of the phase activity coefficient on the concentration, the integration used to derive the constant field equation of Goldman becomes inapplicable in this case. An applicable equation would involve the use of activities and activity coefficients in the original Planck differential equation. The activity coefficient–concentration dependence must then be explicitly inserted in the equation before integration. This has already been done, in one instance, by Linderholm (26), (*cf.* also Helfferich (27)), who stated the activity coefficient variation in terms of a simplified version of the Debye-Hückel formula. One must see whether such integral flux equations will not account for some of the permeability deviations encountered, particularly those expected due to saturation effects. Such agreement with data as is obtained by use of the constant field equation may be largely fortuitous. The disagreement with data, as evidenced by the finding of widely varying permeability coefficients (Hodgkin and Horowicz (4), Sjodin (1), and Adrian (7)) may, on the other hand, have a very good explanation. The permeability coefficient changes observed may just measure the departures to be expected from using an inapplicable equation which does not take enough variables into account.

The taking into account of activity coefficient variation and the assumption of more realistic boundary conditions will not, of course, lead to more applicable flux equations if an insufficient number of driving forces are being taken into account or if the membrane is inhomogeneous. The complications which can occur when the "site density" mentioned earlier is not constant have been emphasized by Teorell (28) who refers to asymmetries of "membrane charge." Of particular relevance are the cases of diffusion with "non-linear adsorption" discussed by Crank (29).

Possible steric factors such as those discussed in detail by Mullins (22) may also provide ample reason for the failure of the simpler flux equations when applied to biological membranes. The present work shows that specific adsorption effects cannot be ignored and also that ion movements can be consistently described, at least in the muscle cell, in terms of specific partition coefficients or affinities. It is noteworthy that a permeability theory put forward by Danielli (30) and extended by Zwolinski, Eyring, and Reese (31) was formulated in just such terms.

If flux equations are to retain generality, they must take account of the competition selectivity discussed. In so far as it is possible to regard the membrane as a macroscopic phase, competition might best be inserted into flux equations as the dependence of the activity coefficient of the jth ion on the concentrations of all other mobile ions in the membrane. If operational flux equations are considered to be special cases of the perfectly general transport equations of irreversible thermodynamics, the competition would be seen to enter as cross-coefficients between each ion and every other ion and the membrane site matrix. The assumption of a macroscopic membrane phase may, of itself, be called into serious question. If ions in cells move in pores of molecu-

lar dimensions where passing is restricted or not possible, it is not clear that any of the existing macroscopic laws would be anything but very rough approximations. For the present, the chemical kinetic or adsorption analog approach used in this work seems to be more useful for many purposes than classical permeability theory which suffers from the lack of equations which take sufficient variables into account.

The treatment used in this work seemingly applies well to the influx of the three ions used. The model applied will be incomplete until the effluxes as well can be related to it in some way. If only potassium ions are considered, the data suggest that most of the results apply to the situation in which potassium influx and efflux are nearly in balance. Since the internal potassium concentration remains essentially constant throughout much of the interval in which uptake rate is measured, it is obvious that efflux cannot obey the same equation obeyed by influx. The following reasoning may indicate how the difficulty may be overcome. The selectivity or affinity constants may be looked upon as partition coefficients by which external concentrations must be multiplied to correspond to concentrations in the outer surface region. If one considers the inner surface of such a region to be in contact with ions at a different electrical potential, it seems likely that this potential would affect the number of ions in the inner surface. This may mean that inside ion concentrations must be multiplied by an additional activity coefficient due to the electrical potential. The magnitude of the electric component of the activity coefficient is given by $e^{\frac{EF}{RT}}$. If inside concentrations are multiplied by this factor, the equation by which efflux would be governed becomes:

$$\phi_{out} = PN \frac{kC_{in}\, e^{EF/RT}}{kC_{in}\, e^{EF/RT} + 1}$$

Together with the equation governing influx, it is obvious that this equation leads to the following relation between C_{in} and C_{out}.

$$C_{out} = C_{in}\, e^{EF/RT}$$

This treatment would then demand that the membrane potential have a value consistent with a system behaving as a potassium electrode. If the same analysis is applied to the bi-ionic cases in which rubidium or cesium ions are present in the external solution, membrane potentials are predicted which are in the correct order, that is, less depolarization being predicted with rubidium ions and still less depolarization with cesium ions. The question then arises as to why influx should be independent of the potential whereas efflux is potential-dependent. The answer might be that most of the potential drop occurs after the outer rate-limiting surface sites have been passed in the inward direction. Outer ions would then only "see" the energy barrier of a layer of sites

initially. Once past the rate-limiting step, they would come under the influence of the potential but would then be counted as internal ions.

These considerations are of a rather empirical nature and the entire influx–efflux–potential system requires a sound physical chemical analysis. It is obvious that simple treatments based on independent ion movements are inadequate since the series of ions studied does not obey the independence principle.

The method of estimating surface concentrations by means of "isotherms" must be regarded as a reasonable approximation. In reality, ions are moving along a gradient of free energy and the problem reduces, formally, to that of finding a potential function that properly represents the free energy in the membrane. If the gradient of the free energy function is taken, a differential equation results which must be integrated across the membrane thickness to yield the flux equation. This will be done in a future publication where concepts derived from the same limited site model will be applied. It will be shown formally that the constant P used in the present formulation is a permeability constant. If the total number of sites N is expressed as a site density having the units of concentration and membrane activity is normalized so as to be expressed in units of concentration, P will have the dimensions of a permeability constant. The numbers representing PN in the present treatment may be taken, then, as relative permeabilities. These permeability coefficients apply only within the membrane and contain no partition coefficients. To obtain the permeability coefficient as defined by Hodgkin and Katz (3), P must be multiplied by the partition coefficient k. Thus, one may write $\bar{P} = P.k$. Values obtained for the ions in this work are presented in Table XII. An equation derived by Hodgkin and Katz (3) from constant field assumptions may be used to estimate the membrane potential, though the equation is now regarded as empirical by Hodgkin and Horowicz (4). The relationship is widely known and is not presented. The equation states, however, that ions in the external solution will depolarize the membrane to extents determined solely by the values of their permeability coefficients, ions with greater permeability coefficients depolarizing the most. Table XII shows that the present theory

TABLE XII

EXPERIMENTALLY DETERMINED RELATIVE PERMEABILITY CONSTANTS

Ion	Relative P	Partition coefficient relative to $k_K = 1.0$	Permeability constant $\bar{P} = p \cdot k$
K^+	133	1.0	133
Rb^+	20	3.6	72
Cs^+	34	0.6	20

would require the three ions to depolarize in the order K > Rb > Cs. The requirement is in qualitative agreement with the findings of Sjodin (1) and of Sandow and Mandel (32) concerning the effects of rubidium and cesium ions on the membrane potential of frog sartorius muscle. If reasonable estimates are made as to the value of the permeability coefficient for chloride ions and the internal chloride concentration, the permeability values in Table XII show good quantitative agreement with the membrane potential data.

When equations such as those presented in the text are obeyed, it has often been concluded that a "carrier" system is in operation and that this system is quite distinct from another membrane process governing the so called passive movement of ions. In the present treatment, the results observed are attributed to a limited number of fixed sites at the outer surface of the membrane. It is not necessary to suppose that two separate systems are in operation nor is it necessary to suppose that permeability coefficients are dependent on the magnitude of a driving force. The permeability constant has been separated into two experimentally discernible quantities, a partition coefficient and a term representing membrane mobility. The same permeability constant describes inward movement over a wide concentration range, competition in bi-ionic mixtures, and predicts the membrane potential to be expected in rubidium and cesium solutions relative to that in potassium solutions.

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