J. Physiol. (1961), **155**, *pp.* 221–245
With 9 text-figures
Printed in Great Britain

KINETICS OF EXCHANGE AND NET MOVEMENT OF FROG MUSCLE POTASSIUM

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(*Received* 9 *May* 1960)

The exchange of the potassium of frog skeletal muscle has been studied over some ten years by means of the isotope ^{42}K (Harris & Burn, 1949; Harris, 1952, 1953, 1957; Keynes, 1954; Carey & Conway, 1954; Hodgkin & Horowicz, 1959). The movement has usually been regarded as being limited by a resistive membrane not possessing any adsorption capacity for the ions. This assumption leads to the use of an exponential law to describe the time course of the process. However, it has been repeatedly found with frog (Harris, 1953; Carey & Conway, 1954) and other muscles (Creese, 1954; McLennan, 1956) that at least two exponential terms are required to fit the whole of the observations. There is initially a more rapid movement than would be expected from the later exponential process; the initial movement lasts some 20 min. When muscles have been loaded with some ^{42}K but full exchange has not been reached, the movement of tracer into an inactive solution having an equal K concentration can only be made to agree with the uptake on the exponential formulation if less than the whole of the muscle K is assumed to participate in the exchange (Harris, 1953).

It has been suggested that some of the anomalies met when applying the exponential formulation to experiments made on whole muscles should be attributed to the effect of extracellular diffusion (Hodgkin & Horowicz, 1959), and it has also been considered (Carey & Conway, 1954; Creese, Neil & Stephenson, 1956) whether variable fibre size could account for them. Computations made from fibre-size data for the whole muscle do not, however, permit the kinetic behaviour to be explained completely on this basis, as has been stated by these authors and by Harris (1957). The effect of extracellular diffusion (as a major factor) has also been dismissed by Carey & Conway (1954), who showed that it could not lead to curves agreeing with their observations. Hodgkin & Horowicz (1959) pointed out, in discussing their single-fibre experiments, that they had not followed the loss of tracer in the early part of the washing-out process; hence there has been no demonstration that one exponential suffices to fit even single-cell results.

In an attempt to fit the kinetic data, Harris (1957) proposed that the movement of the ions was limited by a combination of surface resistance and low internal diffusivity. A modified model is now proposed in which the slow diffusion is limited to an 'outer region' of the cell, which has a definite capacity for K ions determined by its possession of fixed anionic charges. This model allows the kinetic results of K-exchange experiments to be explained. K^+ exchange has to be distinguished from net movement of K^+ which occurs either along with an anion or in exchange for Na^+. By choice of suitable conditions the processes can be measured separately and shown to approximate to the total observed when two take place together.

The laws governing exchange and net movement differ, exchange being a consequence of thermal agitation alone, while net movement takes place down a gradient of chemical potential produced either by adding KCl or as a consequence of metabolic activity. All exchange–time curves can be reduced to a single one by the expedient of plotting amounts of exchange against the product (applied K concentration × time) for a given temperature. This result has already been mentioned briefly (Harris, 1960).

METHODS

The measurements of the uptake of ^{42}K from various media were carried out on sartorius, or (rarely) semitendinosus muscles weighing 50–75 mg, from *Rana temporaria*. It was confirmed in parallel experiments that the two muscles gave similar results so long as their K^+ contents were about equal. The tracer potassium was obtained as bicarbonate, this being used to prepare mixtures having either penetrating anions (chloride) or non-penetrating anions (methyl sulphate, phosphate). The usual chloride-bicarbonate solution contained, in addition to the K^+ concentration specified in the text, (mg ion/l.) Na^+ 110, HCO_3^- 20, Cl^- 94 + number of mg ion K^+ present. Ca^{2+} was added to a concentration of 2 mg ion/l. At the working pH (7·2), bubbling with 95 % O_2 + 5 % CO_2, the HCO_3^- concentration would be in equilibrium with about $1{\cdot}4 \times 10^{-2}$ mg ion/l. of CO_3^{2-} (the second dissociation constant of carbonic acid being taken as $4{\cdot}4 \times 10^{-11}$). This carbonate concentration might be expected to limit the Ca^{2+} concentration to 0·65 mg ion/l. because of the small solubility product of $CaCO_3$ (9×10^{-3} (mg ion/l.)^2), but the precipitation was delayed as long as the solution did not become more alkaline.

Freshly dissected muscles gained up to 10 % in weight after immersion for a few minutes in the mixtures; after this the weight remained constant. It appears that the relative tonicities of the plasma and the saline mixture may be an important factor in determining whether the muscles maintain their K^+ content. A slight reduction of the tonicity reduces the concentration at which the internal K^+ must be held and entails less energy expenditure to bring about a net gain. If the sodium salt concentration was made 120 mM the gain in weight was avoided, but the muscles then gained Na^+ and lost K^+ to media having low K^+ concentrations (Harris, 1957, fig. 7).

In a few experiments solutions having (mg ion/l.) $Na^+ + K^+$ 110, Cl^- 92, HCO_3^- 20 and Ca^{2+} 1 were used. When the K^+ concentration exceeded 10 mg ion/l. in such mixtures there was considerable swelling, which was used to follow the entry of KCl.

To observe the exchange of K^+ in media from which no gain of KCl could occur, solutions of salts of non-penetrating anions were used. The most satisfactory mixture was one

containing the methyl sulphates of Na^+ and K^+ with (mg ion/l.) $Na^+ + K^+$ 110, methyl sulphate anion 90, HCO_3^- 20; Ca acetate = 2 mM. Other solutions used, and referred to in Table 1, were: 'bicarbonate' (mg ion/l.); $Na^+ + K^+ = 110$, $HCO_3^- = 110$, ionized Ca estimated as before 0·01; 'phosphate' (mg ion/l.) $Na^+ + K^+$ 142, phosphate (equimolar $H_2PO_4^-$ and HPO_4^{2-}) 95, ionized Ca 0·03, estimated from the solubility product of $CaHPO_4$.

The tracer entry was found for measured times of immersion in one or other of the solutions made up with a proportion of ^{42}K and was assayed under a Geiger tube. Before each assay the muscle was blotted on clean filter paper previously moistened with an inactive solution otherwise similar to the test solution. In some earlier experiments also used in the results a timed wash of 15 sec had been given before each assay. However, the blotting procedure was found to be less troublesome, and it avoids removing part of the ^{42}K at each assay. The question of whether to include the counting time as part of the exposure to the active solution in the recent experiments was resolved by computing the exchange occurring in 1 min and comparing this with the amount of K^+ carried in an extracellular space amounting to 10 %. With 2 m-equiv K/l. at 20° C the exchange between the eighth and ninth minutes is about 0·28 μequiv/g while extracellular K is 0·2 μequiv/g. Between 24 and 25 min the exchange amount is reduced to about a half, that is, to 0·14 μequiv. In higher K concentrations the ratio (amount exchanged in a given minute interval)/(extracellular K) falls, because (as will be shown) the exchange varies as the root of the concentration but extracellular K is directly proportional to it. Hence we have regarded the counting time as being part of the exposure time. In some experiments, after longer times so that the rate of change of tracer content was low, we made two counts of the muscle, turning it over between each count. Such readings differed by little more than 5 % or the statistical error of the assay.

At the end of each experiment the muscle was divided up and the fragments covered with 1 ml. dilute nitric acid. After digestion the radioactivity was compared with that of a suitable dilution of the soaking solution to establish a cross calibration. The tissue fragments and fluid were then made up to 15 ml. with water and kept for at least 16 hr. The supernatant fluid was analysed by flame photometry for Na^+ and K^+. It was confirmed that this treatment removed all the tissue K^+; it left 1–2 m-equiv Na^+/kg original weight associated with the debris. The analyses are estimated to be accurate to ±2 %.

THEORETICAL

Our results are presented in terms of a particular model which will be described before giving the experimental observations. The model was chosen to explain an empirical relation disclosed when the exchanges were examined over a wide range of concentrations.

The exchange of the cellular K^+ is regarded as a process distinct from that of net movement. The exchange as such can only be measured under conditions chosen so as to avoid net movement. Exchange is supposed to involve two processes; the first is an exchange with ions adsorbed near the cell surface (which may include the reticulum), and the second is diffusion inwards from the source provided by the adsorbed ions at the same time as an equal outward diffusion of the internal ions. The model is essentially a 'three-compartment' one, with the intermediate compartment having ion-exchange properties and within which movement is slow, so that it is the analogue of the resistive membrane.

Let us first examine the kinetics of exchange of ions held in a thin layer and uncomplicated by the presence of an internal reservoir. Suppose there are N sites all holding ions, some of which have been exchanged; these latter are denoted K_a^x, so $N\text{-}K_a^x$ are unexchanged. Given a favourable collision between an ion in solution and an adsorbed ion, an interchange occurs. If tracer is applied at concentration $[K_e^x]$ and the solution is kept free from the desorbed ordinary ions, one can write for the rate of tracer uptake by, say, unit area of the layer

$$dK_a^x/dt = k[K_e^x]\,(N\text{-}K_a^x), \text{ where } k \text{ is a constant.} \quad (1)$$

This simple equation has two interesting properties: first, it integrates to

$$K_a^x = N(1-\exp(-kK_e^x t)), \quad (2)$$

so that the rate constant (kK_e) for equilibration varies linearly with $[K_e]$; secondly, the course of uptake simulates movement through a resistance because it can be written as $dK_a^x/dt = kN(K_e^x - fK_a^x)$, where f is K_a^x/N. If 'f' be regarded as an activity factor acting on the adsorbed ions in proportion to the concentration of K^+ applied, then kN corresponds to a 'permeability', though in fact we have to do with adsorbed ions and not with membrane penetration.

When an ion-exchange layer such as this separates the solution from more ion exchanger, then adsorbed tracer ions can be lost by exchange against non-tracer ions in the next sites. Loss of tracer to the interior will delay the equilibration of the outer adsorption layer. The two extreme conditions which can arise are (*a*) when the outer-layer ions exchange with those in the solution much more rapidly than they exchange with internal ions, and (*b*) when the internal movement is so rapid that the rate of passage through the surface rather than its rate of equilibration determines the progress of the exchange. Case (*b*) is the classical 'resistive membrane' which gives rise to a first-order kinetic law.

The results we obtain, like those of Harris (1953), are not fitted by a single exponential law. They can be approximated by adding two exponentials (Harris, 1953), but turnover in the lower concentrations of potassium does not then appear to apply to the whole of the tissue potassium. This partition into fractions is unnecessary if the consequences of assumption (*a*) are followed, because the course of diffusion *through* the exponentially equilibrated layer requires a series of exponential terms to describe it. Furthermore, the layer provides a region where interference can take place, so that a cell undergoing a net loss of K^+ can maintain a low tracer concentration within its outer region and so impede equilibration of its interior. Another way by which equilibration of the outer layer can be hindered is to add another species of adsorbed ion, such as rubidium. The kinetics of K^+ uptake from $K^+ + Rb^+$ mixtures have been examined and will be described elsewhere, but it is relevant to state here that the additional ions have two effects: (i) they change the rate of equilibration of the

tracer with the surface sites, and (ii) they reduce the total number of sites finally occupied by tracer.

It is useful to examine the applicability of the ordinary diffusion law to movement taking place only by interchanges of position between ions adsorbed on sites in the postulated ion exchanger. Suppose there are N sites per unit volume. At one boundary of a thin layer of thickness δx let the adsorbed tracer concentration be K_a^z so that the local concentration of unexchanged adsorbed ions is $N-K_a^z$. At the other boundary in the direction of normal to the tracer gradient, the tracer concentration will be $K_a^z+\delta x.dK_a^z/dx$, and the unexchanged ions will have concentration $N-K_a^z-\delta x.dK_a^z/dx$. The net rate of movement of tracer across unit area of the layer is given by the difference between the rates of forward and backward interchange. That is:

$$dn/dt = k'.K_a^z(N-K_a^z-\delta x.dK_a^z/dx)-k'(K_a^z+\delta x.dK_a^z/dx)(N-K_a^z) = -k'N\delta x.dK_a^z/dx.$$

Now the velocity constant for interchange, k', operates here for an inter-site separation of δx, which is the distance involved in each interchange. To find the rate of progress, k' can be replaced by a new constant, κ ($\equiv k'\delta x$), giving the constant for interchange over unit length. Then $dn/dt = -\kappa N\,dK_a^z/dx$. Comparing this with the Fick equation for diffusion across unit area we see that κN is equivalent to the diffusivity. Hence we can use the ordinary diffusion laws with constant diffusivity so long as κN remains constant in the ion exchanger.

On the basis of the approximation of the surface build-up of adsorbed tracer to a simple exponential process, it is possible to proceed to examine the time course of diffusion into the cell interior. Diffusion into simple geometric shapes from a *concentration* which obeys the law $C = C_\infty(1\text{-}\exp(-\beta t))$, has been examined by Crank (1956). If his curves are replotted against the quantity $(\beta t)^{\frac{1}{2}}$ it turns out that the progress of equilibration is nearly independent of the internal diffusivity D and the dimensions, provided the ratio $r^2\beta/D$ (r is radius of cylinder or sphere, or half-thickness of sheet exposed both sides) is less than unity. This statement can be confirmed by reference to Fig. 1, in which the curves are replotted from Crank's Fig. 5.4 for the cylinder. The figure shows that changing $r^2\beta/D$ from 0·01 to 0·5 only alters the value of $(\beta t)^{\frac{1}{2}}$ at half equilibration from 0·82 to 0·87, and the slopes of the nearly linear parts of the curves are closely similar. This result makes it appear that when diffusion occurs from an exponentially built-up concentration having rate constant β, the expedient of plotting against some function of βt approximates all curves to one, provided the above restriction on $r^2\beta/D$ is met. To show the independence of shape the following values of $(\beta t)^{\frac{1}{2}}$ at 20 % equilibration may be compared: (*a*) for $\beta = 0{\cdot}1$, sheet 0·50, cylinder 0·49, sphere 0·48; (*b*) for $\beta = 0{\cdot}5$, sheet 0·61, cylinder 0·53, sphere 0·51. At 50 % equilibration the sets of values are even closer. Because D is unimportant (except in determining the restriction on $r^2\beta/D$) we may infer that a system having within the exponentially built-up layer a region with one diffusivity surrounding a further region with another diffusivity will be indistinguishable from one with a homogeneous interior.

According to the model which has been proposed, the value of β will be proportional to the applied concentration $[K_e]$ (since $\beta \equiv kK_e$). Hence the uptakes of radioactivity which measure the progress of equilibration of the nearly constant K^+ content of normal muscles should lie on a single curve when plotted against a function of kK_et. To use the experimental results in this way, the readings of muscle radioactivity were converted to quantities of potassium of specific activity equal to that applied in the relatively large volume of test solution. Variation of external specific

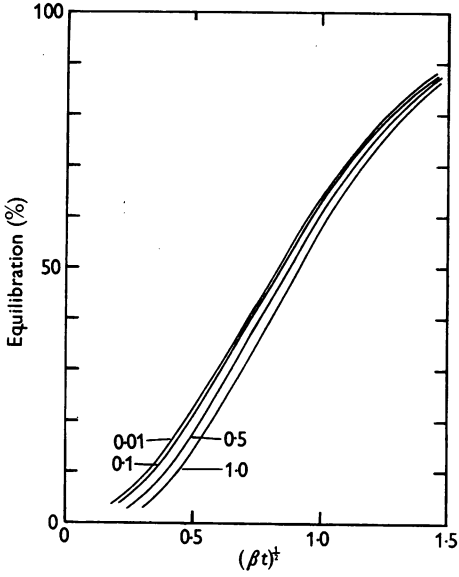

Fig. 1. Theoretical curves for the equilibration of a cylinder by diffusion from a concentration which rises according to the law $C = C_{(t=\infty)}[1-\exp(-\beta t)]$. The numbers against the lines are the ratios $r^2\beta/D$, where r is the radius and D is the diffusivity. Note that the abscissal scale is $(\beta t)^{\frac{1}{2}}$. (Replotted from curves given by Crank, 1956.)

activity during the run was ignored, since it was less than 2 %. The quantities of labelled potassium were divided by the weights of the muscles measured before exposure to the normal saline mixture to obtain contents per gram of tissue. Some variability is introduced here because the ratio of K^+ content to weight depends on such factors as the age and nutritional state of the animal. A starved animal is likely to have muscles with relatively higher proportion of connective tissue than a well-nourished one; on this account the amount of exchange per unit weight in a given interval of ($K_e \times$ time) will be reduced.

RESULTS

Potassium exchange

It is difficult to observe the exchange of potassium without some concomitant net movement. When the external K^+ concentration is reduced to less than 3 mM there is a gradual K^+ loss, and when the applied concentration exceeds about 10 mM a net gain of KCl occurs from chloride-containing media. The exchange measurements made in the media with low K^+ concentrations may have been accompanied by net losses of up to 10 μequiv K/g tissue in an experiment lasting 4–5 hr (see analyses in Table 1). Net gain from the high K^+ media was prevented by making the measurements in solutions of anions which, as judged by their not causing

TABLE 1. Slopes of the linear parts of the K^+ exchange curves obtained when exchange (μg ion/g) is plotted against (K^+ concn. (mg ion/l.) $\times$ time (min))$^{\frac{1}{2}}$. The linear part is considered to be between 8 and 60 μg ion/g exchanged. Final K^+ contents at the ends of the experiments are noted; these have been corrected for the amount of K^+ carried in an extracellular space of 0·1 ml./g

Major anion (mg ion/l.)		K_e	K content (μg ion/g)	Slope	Major anion (mg ion/l.)		K_e	K content (μg ion/g)	Slope
				Experiments at 0° C					
Chloride	96	2	78	0·5	Phosphate	95	4	77	0·6
	98	4	79, 88	0·55		95	8	65	0·55
	102	8	89, 100	0·6		95	25	81·5	0·55
						95	40	81·5	0·5
Methyl sulphate	90	4	64	0·5					
	90	8	77	0·6					
	90	25	88	0·6					
	90	50	94·5	0·5					
	90	100	105, 106	0·55					
Bicarbonate	110	50	92	0·55					
	110	90	99	0·55					
			Mean	0·55					
				Experiments at 20° C					
Chloride	95	1	62	(0·8)*	Phosphate	95	2	60	0·9
	96	2	71, 80	1·0		95	4	64, 61	1·0, 1·0
	97	3	85·5	1·1		95	8	88, 78	1·0, 1·0
	98	4	80, 90, 76	1·1, 1·1, 1·0					
	102	8	90·5, 81·5	1·0, 1·1					
Methyl sulphate	90	2	61, 70	1·1, 0·9					
	90	4	78, 85	1·0, 1·1					
	90	8	90, 72, 72	1·1, 1·15, 1·0					
	90	25	88·5	0·95, 1·0					
	90	50	99	0·95					
	90	100	94·5, 105	0·90, 1·0					
Bicarbonate		1	62	(0·8)*					
	110	100	103, 106	0·9, 1·1					
			Mean	1·02					

* Not included in mean.

swelling when applied as K salts, do not enter the cells. In these media the muscles did show a rapid but limited net K^+ gain which appeared to be the result of displacement of extracellular Na^+. The rapid K^+ movement was complete in a few minutes; it has been demonstrated by transferring a muscle previously exposed to a high concentration of labelled K^+ to a solution having a lower concentration (Harris, 1952, Fig. 1). In what follows it is assumed that the rapid movement corresponds to equilibration

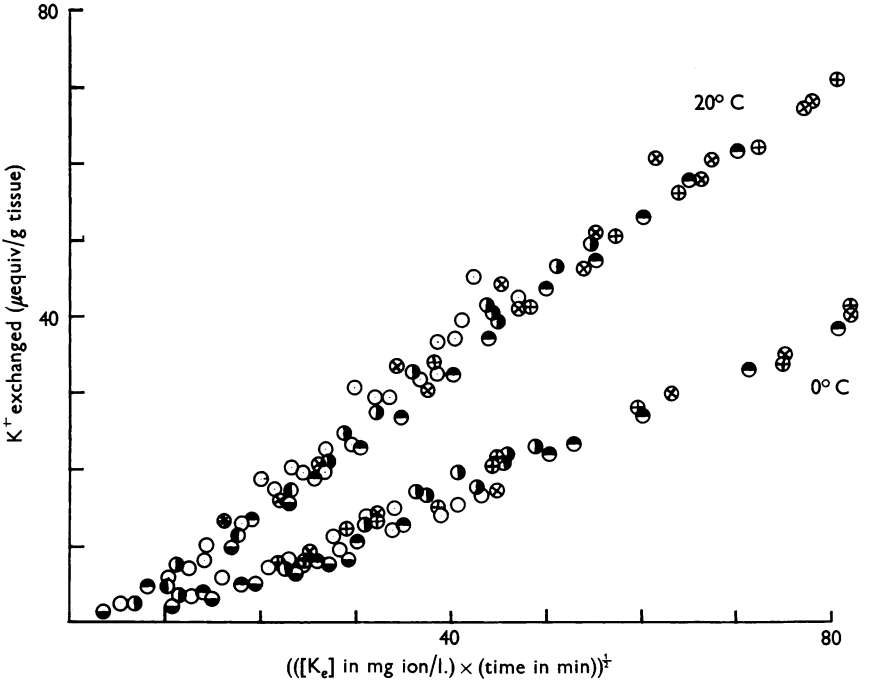

Fig. 2. The exchanges of K observed at 20° and 0° C in solutions with various K^+ concentrations when net K^+ movements were small or absent. The anions used are listed in Table 1. Symbols (numbers are K^+ concentrations in mg ion/l.): ◒, 1; ◐, 2; ○, 4; ◑, 8; ◓, 25; ⊕, 50; ⊗, 100. The K^+ content of the fresh muscles was 85 ± 5 μequiv/g and this figure allows approximate conversion of the ordinates to percentage exchanges.

of an extracellular space equal to 0·1 ml./g tissue. This value is close to values (around 13 %) found by use of inulin (Boyle, Conway, Kane & O'Reilly, 1941; Desmedt, 1953). After subtraction of the presumed extracellular K^+, the amount of labelled K^+ was plotted against (external K^+ concentration × time in min)$^{\frac{1}{2}}$ (Figs. 2 and 3). So plotted, the points for most exchange experiments fell along a single curve for a given temperature. Provided there had been neither gain nor loss of K^+ during the experiment, other results fell on lines nearly parallel to the curve appropriate to the

temperature. By assuming an extracellular space other than 0·1 ml./g tissue such points could be made to fall along the standard curve. The details of the solutions used are given in Table 1, together with final K^+ analyses (adjusted for the extracellular K^+ present in 10% space). In addition, the values are given of the slope of the linear part of the curve obtained by making a separate plot on a suitable scale. The linear part was taken as extending from 8 to not over 60 μg ion K exchanged per gram of tissue. The values for slope are obtained without any deduction of extracellular K^+ from the experimental figures.

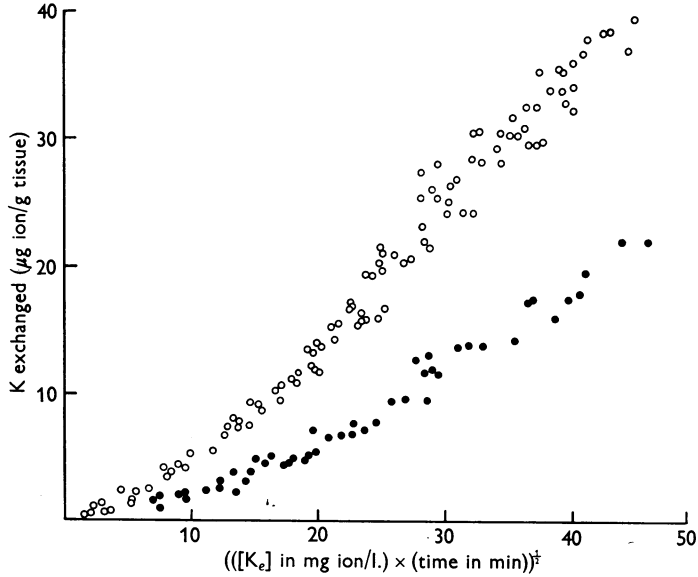

Fig. 3. A similar plot to Fig. 2 permitting use of more experimental points; these were obtained in media with from 1 to 25 mg ion K/l. Points from 2 mg ion K/l. extend to $([K_e] \times t)^{\frac{1}{2}} = 25$, and those for higher concentrations cover the whole range of abscissae. Symbols: ○ at 20° C, ● at 0° C.

Most earlier descriptions of tracer experiments have been presented with logarithmic plots of their time courses. Since we are concerned to show that the exchange is determined only by the product (K^+ concentration × time) at a given temperature, we have plotted on a logarithmic scale $\left(1 - \frac{\text{K exchanged}}{\text{total K}}\right)$ against $K_e \times$ time (Fig. 4). The lines are drawn from the 20° C curves of Figs. 2 and 3, with an indication of the spread of the points. The figure used for total K content was 85 μequiv/g: this was based on 12 analyses of fresh tissue (S.D. ± 5). The higher values of K^+ content in Table 1 associated with immersions in high K^+ concentrations may be explained by there being more extracellular K^+ than was

allowed for in these specimens. It is useful to inquire what effect the extracellular K^+ correction may have had on the logarithmic plot. When up to 8 mg ion K/l. has been used, the extracellular K^+ deduction has not exceeded 0·8 μg ion/g tissue; as compared with the tracer content at the commencement of the linear part of the $([K_e^+] \times t)^{\frac{1}{2}}$ plot of, say, 8 μequiv/g, this is within experimental scatter. On the logarithmic plot this also

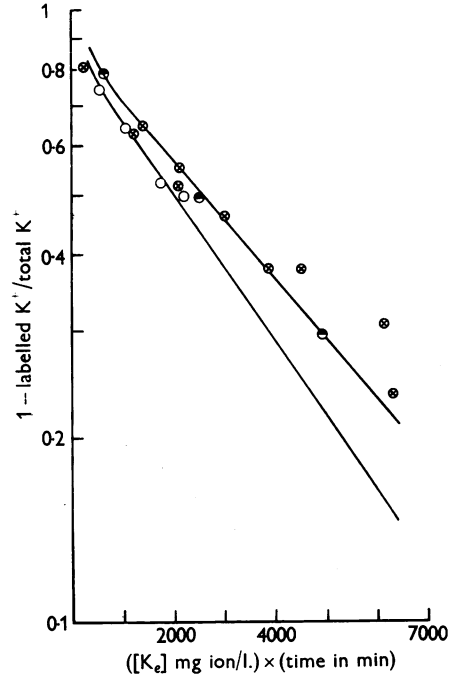

Fig. 4. A logarithmic plot of the 20° C curve of Fig. 2 indicating the spread caused by the scatter of the points. The total K^+ was taken as 85 μg ion/g tissue. The points marked separately are for experiments made with 100 mg ion K/l. (Fig. 6*C*), 25 mg ion K/l. and 4 mg ion K/l. with *no* deduction of extracellular K^+. The K^+ contents, including extracellular K^+, were 113 μequiv/g, 91 μequiv/g and 85 μequiv/g respectively. Symbols as for Fig. 2.

applies to the right of $[K_e^+] \times t = 200$. To take the worst example, with 100 mg ion K/l. present, one can use the (uncorrected) tracer K^+ contents plotted in Fig. 6*C* for exchanges in a non-penetrating anion mixture with the appropriate final total K content (104 μg ion/g), to obtain points indicated on the figure and a few points from other runs, in 4 and 25 mg ion K^+/l. are also included. Evidently the extracellular K^+ does not introduce more error than is to be expected in view of its probable variation between each observation with its associated blotting.

The logarithmic plot becomes linear after the product $[K_e^+] \times$ time

exceeds 900 mg ion/l. × min. Conventionally it has been usual to analyse such time courses in terms of two compartments, either in series or parallel. While our model also involves two compartments, the exponentially equilibrated outer region and the interior, the shape of the curve is determined by the solution to the appropriate diffusion equation and not by the capacities of the compartments, although the latter factor can modify the shape. Our model was selected to explain the observed dependence of exchange on the product $[K_e^+] \times$ time; it would be surprising to find that the K^+ in both compartments of any ordinary two-compartment system shared the property of exchanging to extents respectively determined by this product. It is worth mentioning that the apparent 'fast fraction' of the exponential plot depends as for time course, but not as for amount, upon $[K_e^+]$. One reason for preferring the plot against $([K_e^+] \times \text{time})^{\frac{1}{2}}$ is that this avoids a deceptive division into 'fractions' having unequal exchange rates.

Returning to the results, it must be repeated that when the cell K^+ content at the end of the run was found to be lower than usual, the uptake slope was also often below the average value. It is likely that the slopes listed in Table 1 against 2 mg ion K/l. are low on account of K^+ loss.

The uptake curves of Figs. 2 and 3 only approximate to linearity when the product $([K_e^+] \times \text{time})^{\frac{1}{2}}$ exceeds 20 u. at 0° C and 12 u. at 20° C. This means that to evaluate the slope a certain minimum time must elapse. Comparison of the curves for the two temperatures shows that by making $([K_e^+] \times \text{time})^{\frac{1}{2}}$ at 0° C a factor 1·82 times the value at 20° C, the uptakes become equal within the experimental scatter. For a given concentration the time used at 0° C must be $1{\cdot}82^2 = 3{\cdot}3 \times$ the time at 20° C. The constant 'k' in the equation for surface equilibration is presumably increased 3·3 times by warming to 20° C.

The standard result obtained for exchange in various media allows comparisons to be made of changed conditions; for example, if uptake is carried on serially in media having unequal K^+ concentrations, the results still fall along the standard curve when they are plotted against the square root of the sum of the appropriate ($[K_e^+] \times$ time of exposure) contributions.

Net gain of KCl

The best known method of causing a net gain of K^+ by muscle is to raise the KCl concentration in the external solution (Boyle & Conway, 1941). There is then an entry of KCl tending to bring the values of the products $[K^+] \times [Cl^-]$ inside and outside the cell to equality. Analytical results of the K^+ contents of muscles exposed for various times to raised K^+ concentrations in presence of Cl^- had a wide scatter; they indicated, however, that the uptake became nearly complete within some 40 min

at 20° C with 100 mM-KCl added to Na salts, while with 50 mM-KCl added, the uptake lasted for some 70 min. A more accurate indication of the time course of net change is afforded by use of swelling as a measure of K^+ gain. The swelling which takes place in media having K^+ replacing equivalent Na^+ allows uptake to continue beyond the limit which seems to be reached under non-swelling conditions. The swelling curves for 20° C and 0° C plotted against (time)$^{\frac{1}{2}}$ all have linear portions, but at 20° with 50 mg ion K/l. a limit is reached at 25% weight increase (Fig 5*A*). This limit is surpassed at 0° C (Fig. 5*B*). Boyle & Conway (1941) also noticed that swelling was greater at the lower temperature and ascribed the

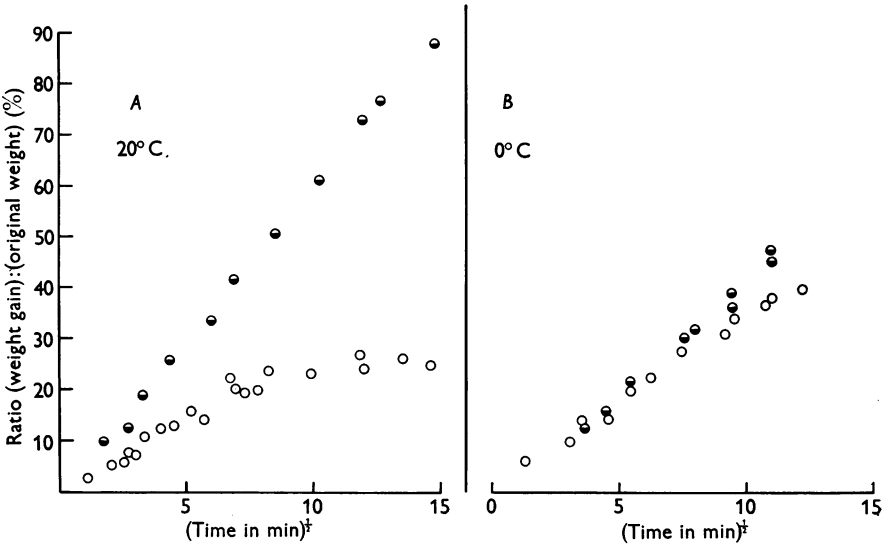

Fig. 5. The ratio (weight gain):(original weight) for muscles immersed in media having either 100 (◒) or 50 (○) m-equiv K^+/l. replacing equivalent Na^+. *A* at 20° C, *B* at 0° C.

difference to a deterioration of the muscle at the higher temperature. If by this one means a loss of internal solutes, the result can be explained. The same effect may be present in the 100 mM-K^+ medium, but it is not seen in the swelling results, perhaps because so much KCl is gained that loss of internal solute is relatively unimportant. Owing to the different behaviours at the two temperatures it is not justified, nor indeed possible, to convert observed swellings into increases in K^+ content by use of a single pair of figures for internal charges and osmoles, as used by Boyle and Conway.

Tracer K^+ uptake when net KCl gain occurs

Tracer uptakes from media containing 25, 50 and 100 mg ion K/l. in presence of Cl^- were measured in a series of experiments shown in Figs. 6 and 7. The figures also show curves obtained in methyl sulphate mixtures from which there is no net gain. The curves are plotted against $(\text{time})^{\frac{1}{2}}$, not $([K_e] \times \text{time})^{\frac{1}{2}}$. Our model, with its ion-exchange outer region, does not allow prediction of the behaviour when the K ions can enter along with co-ions (Cl^-) into the ion-exchange region. The additional K ions could

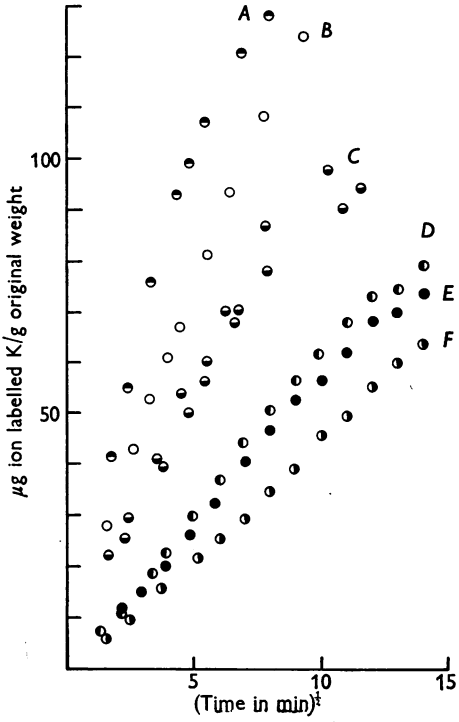

Fig. 6

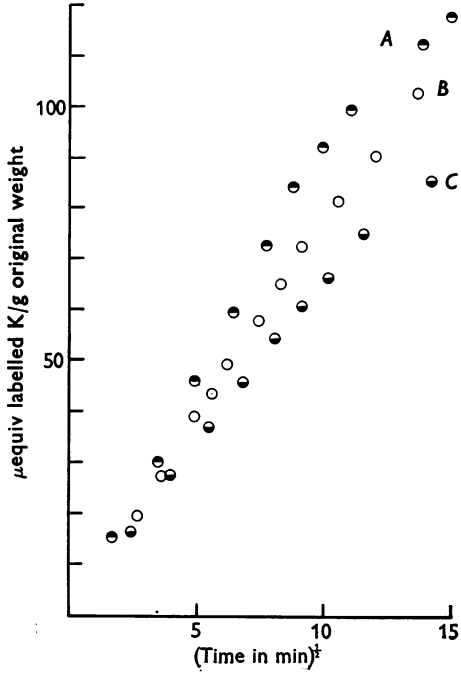

Fig. 7

Fig. 6. Labelled K^+ content–$(\text{time})^{\frac{1}{2}}$ curves for uptakes at 20° C. Curve *A* (◓) with 100 mg ion K/l. and 194 mg ion Cl/l., little swelling; curve *B* (○) with 100 mg ion K/l. and 94 mg ion Cl/l., swelling to 1·5 times original weight; curve *C* (◒) with 100 mg ion K/l. and methyl sulphate anion; curve *D* (◐) with 25 mg ion K/l. and 119 mg ion Cl/l., no swelling; curve *E* (●) with 25 mg ion K/l. and 94 mg ion Cl/l., swelling to 1·1 times original weight; curve *F* (◑) with 25 mg ion K/l. and methyl sulphate anion.

Fig. 7. Labelled K^+ content–$(\text{time})^{\frac{1}{2}}$ curves for uptakes at 20° C. Curve *A* (◓) with 50 mg ion K/l. and 144 mg ion Cl/l., no swelling; curve *B* (○) with 50 mg ion K/l. and 94 mg ion Cl/l., swelling to 1·17 times original weight; curve *C* (◒) with 50 mg ion K/l. and methyl sulphate anion.

either facilitate exchange of the adsorbed ions, or have no effect, or, if they pass through the adsorbed condition, might be part of the stream which normally moves along the adsorption sites. In the latter case tracer entry would remain at the same rate as in absence of KCl gain. It can be seen from the figures that in presence of Cl^- more tracer enters the cell than from methyl sulphate under comparable conditions of time and $[K_e^+]$. With more Cl^- present (the non-swelling media), entry is for some

TABLE 2. Contents of labelled K^+ after exposures to Cl^--containing media and to media having the same K^+ concentration but with non-penetrating anion (n.p.a.) for equal times. The figures (col. 4) are for the terminal points of the curves in Figs. 6 (*A*, *B*, *D* and *E*) and 7 (*A* and *B*) and appropriate interpolations from 6*C*, *F* and 7*C*. The differences between the final K^+ contents determined by analysis are given in col. 6, the content in the n.p.a. solution remained nearly constant. The sum of the net gain and the labelled K content after exposure to the n.p.a. solution is given in col. 7 for comparison with the labelled K content measured after exposure to the Cl^- medium, given in col. 4

Time of exposure (min)	Solution		Labelled K^+ contents		Net K^+ gain in Cl^- mixture	Sum of exchange and gain (5+6)
	K_e^+	Cl_e^-	After Cl^- mixture	After n.p.a. mixture		
	(m-equiv/l.)		(μg ion/g original weight)			
(1)	(2)	(3)	(4)	(5)	(6)	(7)
			At 20° C			
66	100	195	128	78	69	147
224	50	144	118	88	37	125
196	25	119	79	63·5	14·5	78
83	100	94	124	86	62	148
186	50	94	103	84	29	113
196	25	94	74	63·5	14·5	78
			At 0° C			
64	100	194	70	48	31	79
216	50	144	62	56	10	66
170	25	119	43	33	12	55

time greater than from the solution with less Cl^- present (the swelling media). Comparisons between the differences in K^+ content of muscles exposed to the various media and the differences in final tracer K^+ content are presented in Table 2. The analytical difference is subject to uncertainty because muscles exposed to methyl sulphate mixture do not all have the same K^+ content for a given $[K_e^+]$; as mentioned in connexion with the analyses in Table 1, this can be due to different extracellular spaces. This uncertainty in the 100 mg ion/l. mixtures is as much as 10 μequivK^+/g, so it is possible that the results in Table 2 are to be interpreted as meaning that the total tracer uptake is equal to the usual exchange plus the amount of net gain of K^+ with Cl^-. In any case, it is safe to say that the total tracer uptake approaches that expected for normal exchange plus the measured net gain; it may be that a small proportion of the ions which contribute to the net gain use the exchange path and hence reduce the

'exchange' contribution. It is helpful to regard the uptake as made up of contributions from exchange and net gain (along with anion) because this emphasizes the fact that electric charge cannot accumulate in the cell and keeps one aware of the need to consider the forces acting on the ions which must also move when a cation enters. Evidently when a K ion is lost for each one gained the interchange remains uninfluenced by an external electric field; when a K ion moves in with an anion (not, of course, in any bound or un-ionized state) the K^+ movement must depend on the ease of anion movement.

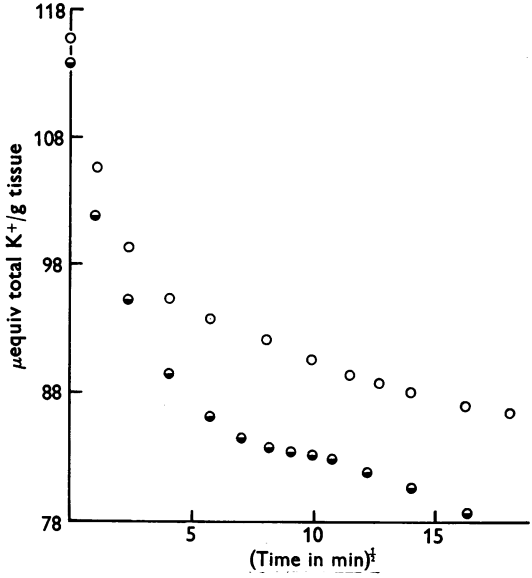

Fig. 8. The time course at 20° C (◒) and 0° C (○) of the net movements of KCl from muscles which had been loaded for 2 hr at 0° C in a mixture having 50 mM-KCl and 110 mM-Na salts. The output curves are reconstructed from sets of analyses; the differences between successive points are accurate to about 5 %, the final points to ±2 μequiv/g. The first point includes the contribution of extracellular KCl.

Net loss of KCl from KCl-loaded muscles

It has been observed that the time required to restore the resting potential of previously KCl-loaded muscles after return to normal saline is strongly temperature-dependent (Grieve, 1960). For this reason, it was thought of interest to examine the dependence on temperature of the KCl loss; the question is whether there is a marked retention of KCl at 0° C. The results given in Fig. 8 show that the K^+ content during washing in saline at 0° C remains about 7 μequiv/g in excess of the value to which it falls at 20° C. A similar result was obtained if the muscles had previously been loaded for 2 hr at 20° C rather than at 0° C but then the 20° C

washing led to a continuing loss of the muscle K^+. That the retention was of KCl rather than of the original K^+ of the muscles was confirmed by chloride analyses. These were made on batches of muscles which had been loaded with KCl as before, but which were then washed in sodium methyl sulphate solution (0·11 M) with 1 mM-Ca acetate. The final Cl^- content of the tissue was found by electrometric titration of a dilute nitric acid extract. Muscles loaded for 4 hr at 0° C and washed for 130 min at 20° C retained only 3 μequiv Cl^-/g, whereas at 0° C they retained 12 μequiv/g. Muscles loaded for 2 hr at 20° C retained 2·1 μequiv Cl^-/g after washing at 20° and 8·6 μequiv Cl^-/g after washing at 0°. The differences between these contents (namely 9 and 6·5 μequiv/g) are similar to the differences in K^+ content seen in Fig. 8.

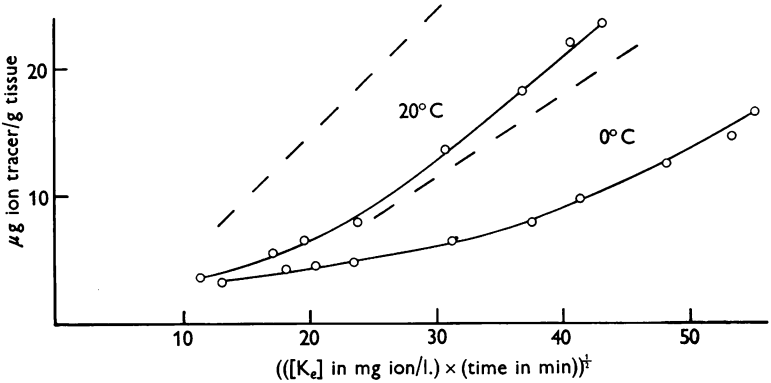

Fig. 9. The uptake of labelled K^+ at 20° and 0° C by muscles which had first been loaded with KCl by exposure to a solution having 50 mM-KCl added to 110 mM-Na salts for 10 hr at 0° C. The dashes indicate where the respective uptake lines running through the points of Fig. 3 would be. In this experiment the external K^+ concentration during exposure to the tracer was 8 mg ion/l.; the anions were methyl sulphate plus bicarbonate.

Tracer uptake when a net KCl loss is occurring

If a muscle is first loaded with KCl by exposure to a mixture containing, say, 50 mM-KCl added to Na salts, and is then put into a solution with a lower tracer K concentration, there is an uptake of tracer along with a net loss of the extra KCl. Under these conditions the tracer uptake is less than the normal exchange amount (see Fig. 9, on which the standard curves are also indicated). After sufficient time has elapsed the tracer uptake curve approaches the usual exchange slope; the extra times required are 30 min at 20° C and 125 min at 0° C in Fig. 9. These times could be equal to the periods during which the major part of the net KCl loss takes place (cf. Fig. 8). This result shows that there is interaction between the net efflux and tracer ions seeking to enter the cell. This apparent contradiction

to the absence of influence of net gain on the exchange process at 20° C already described can be resolved. In the uptake experiments of Figs. 6 and 7 the net gain came about because chloride was present; the rate of collision between tracer K ions and unexchanged sites appears to remain nearly the same at a given K^+ concentration, so build-up of tracer on the sites has the same time course with, or without, the Cl^-, and exchange is unaffected. On the other hand, presence of additional K^+ *within* the cell will increase the number of collisions between emergent ions and the ions on the surface sites, so that adsorbed tracer ions will tend to be displaced back into the solution. Hence, so long as the rate of emergence of ions is abnormally high, the build-up of tracer at the surface will be hindered and uptake slowed.

Tracer uptake when potassium is gained in exchange for sodium

If a muscle is first depleted of K^+ by storage in a K^+-free solution and it is then returned to a medium containing K^+, there is, under some conditions, a net gain of K^+ accompanied by a net loss of Na^+ (Steinbach, 1940, 1951; Desmedt, 1953). According to Carey, Conway & Kernan (1959) and Conway (1960) the Na^+ excretion (and also, we infer, the K^+ gain) takes place either when the Na^+ concentration is reduced from 120 m-equiv/l. during the depletion period to 104 m-equiv/l. during recovery with 10 m-equiv/l. present, or if the external K^+ is raised to over 30 m-equiv/l. at constant Na^+ concentration. The use of high concentrations of K^+ in presence of Cl^- can cause net gain of KCl, and this is not the same as causing the cell to exchange Na^+ for K^+. It seemed desirable to examine the exchange of Na^+ for K^+ under conditions where net KCl gain can be excluded. We carried out both analytical and tracer experiments on muscles which had been K^+-depleted down to contents of between 40 and 60 μequiv/g by storage at 4° C in initially K-free solution with 120 mM-Na salts. After return to a mixture with 3·5 m-equiv K^+/l. and 110 mM-Na salts, the net K^+ gain was $16{\cdot}5 \pm 4{\cdot}0$ μequiv/g tissue in 4 hr (mean and S.D. from 5 paired muscles). With only 2 m-equiv K^+/l. present the change was $5{\cdot}2 \pm 2{\cdot}0$ μequiv/g, which is not significant. No net gains were observed at 0° C, in agreement with Carey *et al.* (1959).

The tracer uptake with 3·5 or 4 m-equiv K^+/l. present in similar experiments was faster than normal, the uptake slopes being between 1·3 and 1·9 (six experiments, mean 1·55) compared to 1·0 for controls (Table 1). Use of 2 m-equiv K^+/l. gave a slope of 1·2 (3 experiments) which does not differ significantly from our standard, although the fact that it is above the average does support the suggestion that, in absence of K^+ loss during the tracer uptake, the exchange slope found in 2 m-equiv K^+/l. solutions would be higher than the values listed in Table 1. At 0° C the slopes found

for depleted muscles were close to the normal value (0·55). It is interesting that the uptakes of tracer by K^+-depleted muscles under those conditions where there was little net K^+ gain, either because of temperature or concentrations used, were close to the normal. If converted to fractional exchanges, as they should be for comparison with fresh muscles, the result implies that the equilibration of the K^+ of the low-K^+ muscles needs less time than does that of the high-K^+ muscles. This would not be unreasonable if the same area of outer region has to feed a smaller total amount of K^+ into the cell interior.

Effects of Na and Ca ions on K^+ movement

At an early stage in this work we thought that Na^+ might be the principal competitor for the surface sites whose occupation by tracer K^+ appears to determine the K^+ exchange (Harris & Sjodin, 1959). However, experiments made in solutions with sucrose replacing part of the sodium salt did not disclose large effects. A difficulty lay in choosing the exact sucrose concentration to cause neither swelling nor shrinkage of the tissue and no net movement. Use of 30 mM-Na salts (20 bicarbonate, 10 chloride) and 6% sucrose led to slight shrinkage; uptake of tracer when 8 mM-$KHCO_3$ was added was the same (within counting errors) as into the paired muscle in a mixture with 110 mM-Na salts. When the same experiment was made with 5·5% sucrose in the reduced Na^+ mixture the slope of the tracer K uptake plotted against $(K_e \times t)^{\frac{1}{2}}$ was 1·1 in the low-Na^+ and 0·85 in the normal solution. However in this second experiment the muscle from the normal solution contained less K^+ at the end of the run than did the other, so it is likely that the lower slope should be ascribed to an effect of K^+ loss. Earlier experiments, mentioned by Harris (1957), in which periods in low-Na^+ mixture were interposed between periods in normal solution, failed to show any change of slope. The importance of tonicity in determining the retention of K^+ by the cell has been shown analytically; for example, by Shaw, Simon, Johnstone & Holman (1956, Table 7). It appears that the tracer-K^+ uptake affords a sensitive indication of net K^+ loss, since in several experiments in which a change was made from 110 mM-Na salts to 120 mM-Na salts during exposure to a constant tracer-K^+ concentration, there was a slowing of uptake after the change. More extreme increase of Na salt concentration led to a fall of tracer-K^+ content and analysis confirmed that a net K^+ loss had occurred.

Another possible competitor for adsorption sites is calcium. It is doubtful whether the concentration of Ca ions is increased when more Ca salt is added to the solutions containing 20 m-equiv/l. of bicarbonate ion, so tests of 10 mM of Ca acetate added to methyl sulphate mixture were made with only 3 m-equiv/l. bicarbonate present.

Three double experiments on paired muscles in either Ca^{2+}-free solutions or solutions with 1 or 2 mM-Ca salt showed no significant difference in the tracer-K uptake slope; a feature common to all experiments was that the line in Ca^{2+}-free solution was displaced towards the origin, as if the muscles had gained 2–4 m-equiv/g more of a rapidly equilibrated fraction of K^+. The effect of adding 10 mM of Ca acetate (as compared with 10 mM-Na acetate to the control) was to cause a slight diminution of the exchange slope—from 1·3 to 1·1 and from 1·0 to 0·8 in the two pairs of experiments. In these experiments all the muscles gained less than 5 % in weight, but those from the high-Ca^{2+} solution had 4 μequiv K/g tissue less total K at the end of the experiments than did the controls. Similar experiments made in the chloride-bicarbonate media with either 2 or 10 mM-$CaCl_2$ showed comparable differences in slope. Accordingly it appears that sufficiently raised Ca^{2+} has some slight hindering effect on K^+ exchange irrespective of the bicarbonate concentration, but it is not excluded that the effect is attributable to a displacement of some of the muscle K^+ by Ca^{2+}.

DISCUSSION

Comparison with other models

The usual concept of a cell supposes a thin, highly resistive membrane of negligible capacity for substances bounding an interior within which diffusion is rapid compared with the rate of membrane penetration. This model leads to the first-order (logarithmic) law holding for the uptake of a substance from a fixed external concentration, but sufficiently early in the process the amount in the cell would be proportional to the product: $K_e^+ \times$ time, where K_e^+ is the applied concentration. After longer times the simple theory leads to the fractional amount exchanged being given by 1-exp(-kt), where k depends on the membrane permeability. As already mentioned, it is necessary in practice to use two or even three exponentials to fit the observations (Harris, 1953; Troshin, 1960). Part of the cell K^+ seems to exchange very slowly or not at all in media of low K^+ concentration.

Attempts have been made to apply the constant-field equation (Goldman, 1943) to the quantitative interpretation of tracer movements. The theory assumes independent inward and outward movements of the ions, so no competition for occupation of adsorption sites would be permissible. It may, however, be of interest to calculate the relative ion movements expected from some chosen external K^+ concentrations by means of this approach. As usually written the equation involves the external-internal potential difference V, but under exchange conditions the value of V must be the equilibrium potential for K ions, equal to $\frac{RT}{F}\ln\frac{K_i}{K_e}$.

Making this substitution one obtains

$$\text{K influx} = P_{\text{K}} \frac{\text{K}_e.\text{K}_i}{\text{K}_i - \text{K}_e} \ln \frac{\text{K}_i}{\text{K}_e},$$

where P_{K} is a permeability constant for potassium.

Let us use this to find the relative times for a given small entry of tracer (small so that its efflux can be ignored). With K_i = 140 m-equiv/l. and K_e = 2, 10 and 100 m-equiv/l. the times stand in the ratios 1:0·3:0·07. The relative values of the times at 20° C for a certain exchange in each of the three media are readily calculated from our empirical relation between exchange and the product ($\text{K}_e \times$ time); equal amounts of exchange in the three concentrations above will be attained after times standing in the ratios $\frac{1}{2}:\frac{1}{10}:\frac{1}{100}$ i.e. 1:0·2:0·02. The constant-field values do not agree with the experiment. It is necessary to make the permeability P_{K} an empirical function of K_e (or potential difference) to obtain a fit. The theory does not lead to the prediction of behaviour under changed conditions; for example Sjodin (1959) had to make P_{K} a function of the composition of the solution when Rb or Cs was present.

An indication that a simple model is inadequate to simulate the behaviour of the cellular potassium was obtained by Harris & Steinbach (1956) by applying water or sucrose extraction to muscles first exposed to tracer K^+. It was found that the specific activities of the K^+ in the extracts fell progressively; either separate fractions of unequal lability exist or the internal mixing is slow.

The present model attributes to the membrane, which may include the reticulum, an important capacity for ions. Comparison between the curves of Figs. 1 and 2 leads to an estimate of about 10 μg ion/g being accommodated in the adsorption region; a better inference of the quantity has been obtained from the Rb experiments. Diffusion into the rest of the cell from the ion-exchange surface may all be a matter of intersite exchanges, or in some parts free diffusion may occur. Given that the outer part of the adsorption material has a constant capacity for K (and K-like) ions and that interchange depends on collision rate, hence on concentration in solution, our first result is explicable. This contrasts with the law governing diffusion into a volume of ion exchanger or water where a given, constant, concentration is applied throughout the diffusion; under these conditions the amount entering is initially proportional to the product (concentration applied) $\times$ (time)$^{\frac{1}{2}}$, a relation used for example by Eggleton, Eggleton & Hill (1928) to relate the quantity of lactic acid diffusing into or from muscle with time and concentration. Strictly, the simple relation holds for a semi-infinite volume. In some model experiments made on tracer K^+ uptake by rods of phenol sulphonic resin (kindly provided by Dr Kitchener

of The Imperial College) it was confirmed that K^+ uptakes over an eight-fold range of concentration did all fall initially on one line when plotted against concentration $\times$ (time)$^{\frac{1}{2}}$.

In the muscle model the slow exchange is presumably due to the slow build-up of the surface concentration, though this may also be so low that it restricts the exchange rate. The value of the internal diffusivity, and even the geometry of the interior can be unimportant provided $a^2\beta/D$ is less than unity. If the iontophoresis result of Harris (1954) is used to deduce the internal K diffusivity, then for a cylinder of radius 40 μ β has to be less than 0·5 sec^{-1}. The slight effect of extracellular diffusion implies in fact that β is less than 0·01 sec^{-1}.

It is important to note that the linear portions of the theoretical curves (Fig. 1) do not extend to the commencement of the exchange, so arguments against the applicability of a diffusion law should not be based on a non-linear dependence of uptake on (time)$^{\frac{1}{2}}$ early in the experiment (cf. Hodgkin & Horowicz, 1959). The curves of Fig. 1 are obtained by adding a series of exponential terms. As t increases, the relative importance of all but one term falls and so eventually the process can be exactly fitted by a single exponential function. The coefficient of this major term is, however, not equal to the amount of material exchanging and the intercept with the ordinate of the linear portion of the function when it is plotted logarithmically is less than the 'cellular potassium'.

The present model has been evolved from the surface-resistance–diffusion model discussed by Harris (1957). Its advantages are that it explains the interdependence entailed by that model between the surface resistance, the diffusivity and the applied concentration. Since the present model involves only the time constant of build-up of tracer K^+ in the surface region, which can depend linearly on the applied concentration, it is not necessary to suppose that the internal diffusivity undergoes changes. The shape of the curves, which on the resistance–diffusion model varied with the ratio of resistivity to diffusivity, is with the present model practically independent of the ratio between time constant for surface build-up and internal diffusivity. Hence, instead of requiring permeability and diffusivity to vary proportionately to maintain a constant shape of curve, no special requirement is imposed. It is to be emphasized that in this paper we suppose that it is the rate of build-up and not the final amount of K^+ attached to the fixed sites of the outer region which varies with the applied K^+ concentration. This would follow if the outer region had a fixed capacity for K (or K-like) ions. Our finding that net KCl gain merely adds to the normal exchange indicates that the extra K ions entering with Cl^- do not increase the rate of equilibration of the outer region, rather they may diminish it.

It is evident that a true exchange process should be uninfluenced by electrical gradients, as was pointed out by Ussing (e.g. 1949) in his discussion of 'exchange diffusion'. Our exchange results bear this out, the internal–external potential difference of the muscle diminishes from 90 mV to close to zero as the K^+ concentration is raised, but the exchange plotted against a function of (K concn. × time) remains constant. Fewer assumptions are needed to explain this empirical relation between exchange and K^+ concentration than when attempts are made to relate independent in- and out-fluxes to permeability factors.

Unlike the K^+-for-K^+ exchange, the net movements of the ions must usually be influenced by, as well as set up, electrical gradients because of the unequal mobilities of most ions. As example, the Cl^- ion is normally nearly excluded by the negativity of the muscle interior; as the potential difference, which reflects the K^+ selectivity, is reduced the Cl^- enters in company with additional potassium. The presence of a reservoir of material instead of a resistive membrane between the inside and outside of the cell will not change the usual physico-chemical considerations such as the Donnan distribution and potential difference. The intermediate region would, however, allow the explanation of K^+ accumulation in the interior as being brought in by the selectivity of the region alone rather than of the whole cellular contents. The requirement that the ions move either in exchange for others of the same charge or along with ones of unlike charge entails interdependence between the Na^+ output, Cl^- gain, and K^+ uptake when a cell is gaining or losing K^+. It may be recalled here that the accelerating effect of externally applied K^+ on Na^+ output is best seen if the muscles have initially raised Na^+ content; that is, just the condition under which a net gain of K^+ and loss of Na^+ can take place.

Interaction in tracer experiments

Under all conditions which we applied, the K exchange component was the major factor in determining tracer uptake. For this reason tracer experiments are unfavourable as a means of detecting small net gains of K^+. On the other hand, the occurrence of a net loss of K^+ is made more evident on account of the expulsion of tracer from the outer region by emerging unlabelled ions. Thus a net loss can give rise to a disproportionate effect on K^+ uptake. A case of net K^+ loss giving rise to slower K^+ uptake has been described by Harris (1957), where the K^+ loss was caused by strophanthin.

Extracellular diffusion

The slower the rate of build-up of tracer concentration in the outer, adsorption, region the less will the observed rate of K^+ exchange depend on the delay imposed by extracellular diffusion. In their original analysis

of the hindering effect of extracellular diffusion Harris & Burn (1949) used the classical resistive membrane model and estimated that a sartorius (thickness 0·6–0·9 mm) when exposed on one side would take about 1·5 times as long for a given exchange as would its individual fibres; the factor was about 1·15 when both sides were exposed. Some confusion has been caused because in that paper the equations were written with a diffusivity (D/λ^2) which included the fractional extracellular space implicitly. The mean value of the space used was 0·19. When account is taken of this the diffusivity of Na^+ in the extracellular space turns out to be close to that in free solution.

Support for the contention that the effect of extracellular diffusion in the sartorius is small is obtainable by comparing the results of Keynes (1954) for whole muscle, which had an exponential exchange constant of 0·088 hr^{-1} in 2·5 m-equiv K/l. at 16° C with both sides exposed, or 0·078 hr^{-1} at 19° C with one side exposed, with the exponential rate constants of between 0·06 and 0·15 hr^{-1} for single muscle fibres, reported by Hodgkin & Horowicz (1960). The exponential portion of our exchange curve (Fig. 4) corresponds to a rate constant in 2·5 m-equiv K/l. of 0·04 hr^{-1}. Since, however, the exponential only applies to part of the total K, it is preferable to compare measured uptakes into muscle with those into single fibres. Using the values given by Hodgkin & Horowicz (their table 6) for various exposures to 2·5 m-equiv K/l. solution the appropriate figure for $([K_e] \times \text{time})^{\frac{1}{2}}$ allows a value to be interpolated from our Fig. 3. The pairs of values are as follows:

(*a*) 113 μ fibre at 32·6 min had 2·34 μequiv K exchanged/ml. myoplasm; $([K_e] \times t)^{\frac{1}{2}} = 8{\cdot}95$, whole-muscle exchange about 3·8 μequiv/g;

(*b*) 98 μ fibre at 10 min had 1·49 μequiv K exchanged/ml. myoplasm; $([K_e] \times t)^{\frac{1}{2}} = 5$, whole-muscle exchange about 1·8 μequiv/g;

(*c*) 90 μ fibre at 60 min had 9·64 μequiv K exchanged/ml. myoplasm; $([K_e] \times t)^{\frac{1}{2}} = 12{\cdot}2$, whole-muscle exchange about 6·6 μequiv/g;

(*d*) 137 μ fibre at 124 min had 7·34 μequiv K exchanged/ml. myoplasm; $([K_e] \times t)^{\frac{1}{2}} = 17{\cdot}6$, whole-muscle exchange about 10·5 μequiv/g.

These comparisons of experimental values show that turnover in the larger single fibres is about the same as the mean exchange in a whole muscle. Remembering that the mean diameter of the fibres in the sartorius is 80–100 μ it appears that the hindrance imposed by extracellular diffusion is compensated by the presence of the smaller fibres so as to make the result similar to that holding for a large single fibre.

SUMMARY

1. The exchange of the cellular potassium of frog muscle follows a unique course, at a given temperature, if plotted against a function of the product (time) × (external K^+ concentration).

2. When the function in (1) is made the square root, much of the exchange is linearly related to it.

3. The exchange at 0° C becomes equal to the exchange at 20° C if the product (time) × (concentration) at 0° C is made 3·3 times the value of the product at 20° C.

4. The exchange may be slightly impeded by Ca ions, but Na ions have little or no effect. It is important to avoid net changes of K^+ content when testing for effects on exchange.

5. When the K^+ concentration is raised in presence of Cl ions there is a net gain of KCl depending on the gradient of the product $[K^+] \times [Cl^-]$. During net K^+ gain at 20° C the tracer-K^+ uptake approximates to the sum of normal exchange plus net gain.

6. If K^+-depleted muscles are exposed to tracer-containing media from which they gain K^+ (by interchange with cellular Na^+), the tracer uptake exceeds that found under exchange conditions.

7. Tracer-K^+ uptake is delayed while a net loss of K^+ from the tissue is in progress.

8. The results can be explained if K^+ enters the cell from an outer region of fixed K^+ capacity which equilibrates with the external K^+ exponentially and with rate constant proportional to the external K^+ concentration.

Our thanks are due to Mrs V. Bolingbroke for her careful assistance.

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