J. Physiol. (1952) 117, 278–288

THE EXCHANGEABILITY OF THE POTASSIUM OF FROG MUSCLE, STUDIED IN PHOSPHATE MEDIA

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(*Received* 29 *October* 1951)

The accumulation of potassium in muscle cells may either be the result of physical forces derived from the existence of the membrane potential (the Gibbs-Donnan effect) or it may be brought about by the intervention of chemical combination. The latter might conceivably be a process involving groups in the membrane itself, as suggested by Lundegårdh to account for the uptake of ions by plant roots.

Preliminary results of the rate of potassium turnover in frog muscle were obtained by Harris & Burn (1949) by using the radioactive isotope ^{42}K. It has since proved difficult to extend the results in a satisfactory way because, in saline media, a net loss of potassium so often takes place (Fenn & Cobb, 1933; Horton, 1930). In the experiments reported here the turnover has been followed in high concentrations of potassium, and potassium is gained rather than lost. These appear to furnish an unexpected result. At 18° C the intracellular K is all equally free to exchange with the external K, while at 0° C some part of the internal K exchanges much more slowly than the remainder. This observation is discussed in relation to the membrane potential measurements of other authors.

It is well known that muscles placed in high potassium media swell if an anion such as chloride, which can also enter the cells, is simultaneously present. To avoid swelling, phosphate solutions were employed.

EXPERIMENTAL

Materials

The sartorii of *Rana temporaria* were used. Where stated, the muscles were employed with a portion of the pelvic bone still attached in order to minimize damage. The radioactive potassium was obtained in the form of carbonate from A.E.R.E., Harwell.

Methods

In order to follow the uptake of potassium, a solution containing a proportion of ^{42}K was prepared. The solution was 0·1 M with respect to phosphate, and contained 140–150 m.equiv potassium per litre, with a resulting pH of 6·8–6·9. This preparation was used as the standard of

radioactivity in order to relate a certain number of counts/min with a known amount of potassium; it was thus possible to convert counts/min to μequiv of potassium derived from the solution. Potassium of standard radioactivity will be distinguished by the symbol ^{x}K.

The muscle or muscles were either (*a*) tied to a frame in order to maintain a constant geometry throughout the experiment, or (*b*) furnished with cotton loops which could be fitted when desired between two pegs in a plastic base, so that the specimens could be slid beneath a Geiger counter in a fairly reproducible position. Method (*a*) better maintains geometry, but has the disadvantage that the frame, being exposed to the solution during the experiment, takes up a certain amount of ^{x}K. The muscle, with its frame or loops, was exposed for measured time intervals to the test solution, and the radioactivity measured after each period. In order to remove adhering and some extracellular fluid a timed wash was given before each assay. The wash solution employed was either saline (0·65% NaCl, 0·02% each KCl and $CaCl_2$) or 0·1 M-sodium phosphate, pH 6·9. There was no obvious difference in result between the two solutions. The duration of the wash was generally 3 min. At the end of the experiment the muscle was dissolved in a few drops of nitric acid and an aliquot of the diluted muscle solution was counted. The same counter was used for assay of a dilution of the ^{x}K solution. This provided the relation between quantity of ^{x}K and radioactivity.

In order to determine the rate of ^{x}K loss from a muscle, muscles were prepared in one of two ways: either (*a*) an injection of 0·5 ml. 0·1 M-^{x}K phosphate was administered to a frog on the day before the experiment, or (*b*) the muscles were soaked in a solution of ^{x}K phosphate.

During measurement of ^{x}K loss (which may either be a net loss or an exchange of ^{x}K for ordinary potassium derived from the external solution) the adhering solution, once the run was started, was not appreciably radioactive, so no washing was given.

The ^{x}K used for injection had an activity of 2–8 mc/g. That used for phosphate solutions was diluted 50-fold when used on the day of receipt, and correspondingly less as decay reduced the activity.

The radioactivity readings were corrected for decay, background, and resolution time of the scaler-Geiger counter combination.

The volume of liquid used in the runs was about 50 ml. Even with the lowest potassium concentrations employed this contains at least 50 times as much potassium as the muscle, and the stronger media had some 500 times as much. No great error is therefore introduced if the external phase is regarded as of constant composition. In some experiments the liquid was kept agitated by introduction of oxygen through a sintered glass disk; this had no obvious effect on the results.

RESULTS

Extracellular potassium and its diffusibility

An account of the complication introduced into the study of the permeability of those cells which are only available as a compact bundle has been given by Harris & Burn (1949). The fact that the ions in the medium have to diffuse through more or less extracellular fluid before they reach the cells introduces a delay, and indeed the rate of diffusion may set the limit to the rate of uptake. This is particularly the case when the specimen is thick, and when the cells have a high permeability. When thin specimens are employed a correction can be applied for the diffusion delay. In order to see whether diffusion was likely to introduce a serious delay into the K turnover between cells and medium in this particular case, it was necessary to be able to observe the two processes separately. This was not possible in the previous work in which saline media were used, but the application of potassium phosphate media has permitted the distinction to be made.

Muscles were soaked for a few hours in ^{x}K phosphate solution, then tied to a frame, assayed, and plunged into a saline medium made up with ^{x}KCl (2–12 m.equiv/l.), NaCl 0·65 %, $CaCl_2$ 0·02 %. The result is an outrush of the extracellular ^{x}K phosphate, in exchange for the ions in the saline (Fig. 1). At the same time intracellular potassium may begin to increase or decrease its specific activity, depending on whether the value was lower or higher than that of the saline potassium. In addition, some net loss of potassium occurs, because

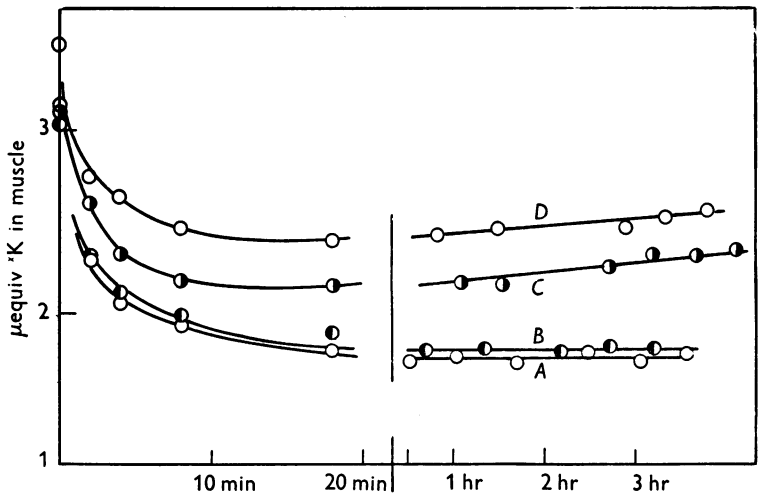

Fig. 1. The time course of the ^{x}K content of muscles which were prepared by treatment with ^{x}K phosphate (specific activity (S.A.) 1 arbitrary unit) and were then put into saline solution containing ^{x}KCl (S.A. 1) at 18° C. The preliminary soaking had given the internal K of the muscles S.A. 0·33 (*A* and *B*), 0·4 (*C*), and 0·5 (*D*). The initial rapid loss of ^{x}K, presumably from the extracellular spaces, is followed by a period of practically constant ^{x}K content. This happens because the net loss of internal K having S.A. 0·33 to 0·5 tends to be balanced by a further exchange between internal and external potassium, and the latter has S.A. = 1. In the two higher concentrations of ^{x}KCl this additional exchange can be seen to be leading to a gradual increase in the ^{x}K in the muscle. The solutions used for the four runs were: *A*, 1·84 m.equiv K/l.; *B*, 3·68 m.equiv/l.; *C*, 7·36 m.equiv/l.; *D*, 11·04 m.equiv/l.; all with 0·65 % NaCl and 0·02 % $CaCl_2$.

the cells do not maintain so high a concentration of internal potassium in the saline as in the phosphate. Accordingly, there is a slow change of cell radioactivity due to adjustments of quantity and specific activity of the cell potassium. In the runs illustrated the effects nearly cancel out, making it easy to evaluate the rapidly lost ^{x}K and the time scale of its exit.

The mean volume of the extracellular fluid relative to the muscle volume was deduced from the quantity of ^{x}K lost rapidly, and came to $0{\cdot}19 \pm 0{\cdot}04$ (S.E., six experiments); the time for loss of half the fast moving ^{x}K was 1·2–1·5 min at 18° C from muscles about 0·8 mm thick exposed to the solution on both sides. From these figures it appears justified to neglect the effect of

diffusion upon the observed rate of turnover of potassium between cells and solution, for which the half-time at 18° C is over an hour.

The efflux of ^{x}K *at* 18 *and* 0° *C*

The efflux of ^{x}K in exchange for ordinary potassium derived from the medium was followed at 0 and 18° C. The solution contained 140 m.equiv K/l. After correction for decay, etc., the quantities of ^{x}K remaining in the muscles, relative to the initial quantity, were plotted as a function of time (Fig. 2).

Both at 0 and 18° C there is first a rapid loss of ^{x}K, some of which is derived from the extracellular spaces. At 18° C, the loss then follows an exponential course certainly down to a ^{x}K content of 10 % of the original. At 0° C, however, the behaviour is more complicated, for the loss did not become exponential, and when some specimens were left in the solution overnight a considerable (15–25 %) proportion of the ^{x}K was retained in them. Fig. 2*a* is a continuation of one of the curves of Fig. 2 and shows that between 21 and 25 hr the fraction ^{x}K (at time t)/^{x}K ($t=0$) only fell by a few per cent, an exact measure of the rate not being possible on account of the errors introduced by the statistical nature of the assay and the imperfect reproducibility of the muscle geometry with respect to the counter. Similar behaviour was found in runs made with, or without, stirring, and both when using muscles from frogs injected 16 hr before dissection (4 runs) and when using a muscle prepared by exposure to ^{x}K phosphate for 2·5 hr at 18° C. Cessation of movement of the ^{x}K cannot have been caused by the ratios ^{x}K/K becoming equal in cells and medium because there was some 500 times as much potassium in the solution as in the muscle; thus equilibration of internal and external specific activities would only be reached at ^{x}K/K = 1/500. The decay period of the radio-potassium retained in the muscle was checked and it agreed with that of ^{42}K. Although this test is not sensitive to ^{24}Na, which has a rather similar decay period, the sodium is known to be rapidly lost from muscles at 0° C to a potassium phosphate medium (Harris, 1950). When the operating temperature was changed, either after 5 hr (Fig. 2) or 22 hr (Fig. 2*a*) from 0 to 18° C, the rate constant of the process rose to about the same value as that found in experiments made entirely at 18° C.

The results can be summarized by stating that, at 18° C, about 70–80 % of the potassium exchanges with a rate constant of 0·45–0·48 hr^{-1}, the remainder exchanging more rapidly; whereas at 0° C there are three fractions, one which exchanges very slowly (15–25 %), one exchanging with a rate constant of about 0·2 hr^{-1} (60 %) and the remainder being more rapidly exchanged.

The influence of potassium concentration on the exchange

Concentrations of potassium other than 140 m.equiv/l. were used in some runs at 18° C in which the efflux of ^{x}K was observed. Mixtures of mono- and

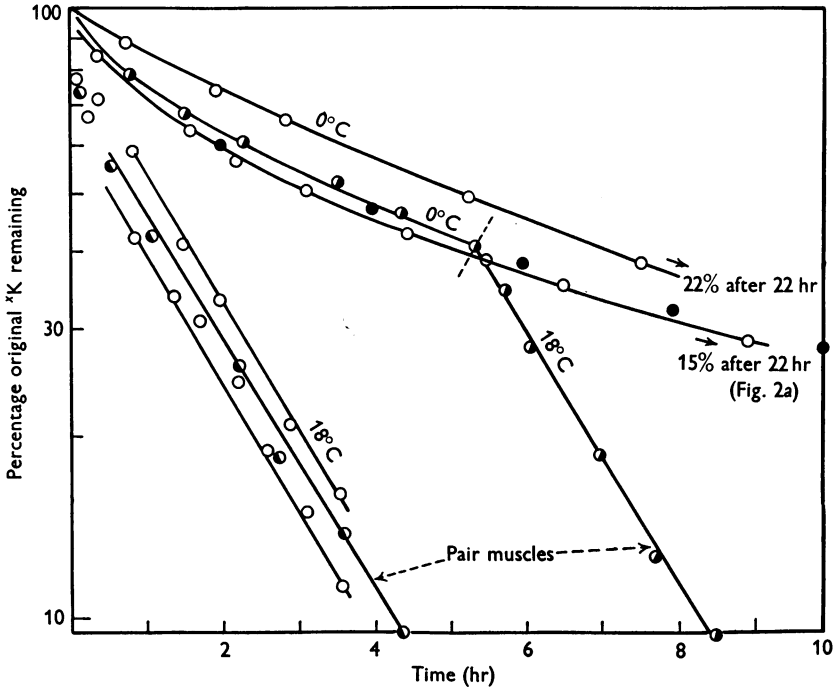

Fig. 2. The time course of the loss of ^{2}K to 0·1 M potassium phosphate at 0 and 18° C (note logarithmic scale). In one run the temperature was changed during the course of the experiment. ●, points calculated from $^{2}K/^{2}K$ $(t=0)=0{\cdot}6 \exp(-0{\cdot}2t)+0{\cdot}2$, to show that at 0° C, after 1 hr the loss of the ^{2}K can be fitted by such an equation, using suitable coefficients for the individual curves. Three runs at 18° C, and three starting at 0° C are shown. The muscles are from frogs previously injected with ^{2}K phosphate.

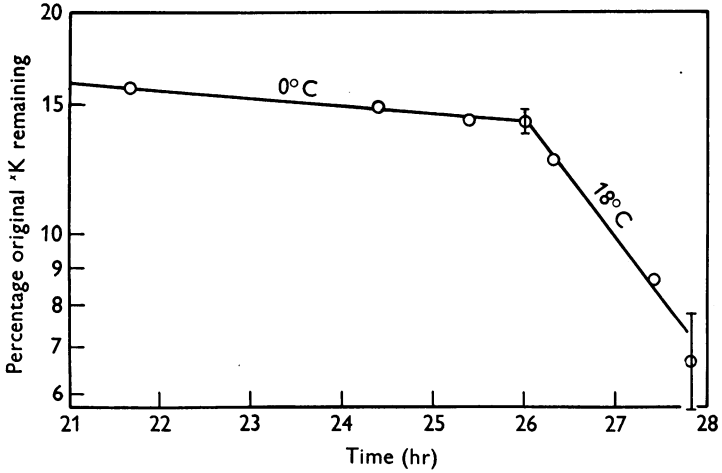

Fig. 2*a*. Continuation on the same scale of one of the 0° C runs of Fig. 2 on the following day. The slow rate of loss at 0° C, and the increase which takes place upon warming to 18° C can be seen. Towards the end of the experiment the error in estimation of ^{2}K becomes serious, as shown by lines representing +S.E. The earlier observations are limited in accuracy by reproducibility of the muscle geometry with respect to the counter, which is estimated to be to within 5 %.

di-basic potassium phosphate were used for 140–184 m.equiv/l. potassium concentrations, and to obtain lower concentrations mixtures of di-basic phosphate with appropriate proportions of sodium phosphates were employed. The pH of the 184 m.equiv/l. solution was 8, whilst that of the other mixtures ranged from 6·8 to 7·4. The rate constant of the loss of ^{x}K was not appreciably altered when the external potassium concentration varied between 100 and 184 m.equiv/l. It did, however, show a diminution when the concentration was reduced to 37·5 m.equiv/l. (0·21 hr^{-1}) and it was still lower in a solution containing 18 m.equiv/l. (0·12 hr^{-1}).

^{x}K *entry from phosphate media at* 0 *and* 18° *C*

When muscles are exposed to a solution of ^{x}K phosphate there is a rapid uptake of ^{x}K. As the final potassium content after such treatment was generally in excess of the potassium content of freshly dissected muscles it is not always accurate to speak of an exchange (of ^{x}K for ordinary potassium); there can be a net gain of ^{x}K, perhaps in exchange for the intracellular sodium.

As mentioned in the outline of methods used, a timed wash was given to the muscle before each assay of the content of ^{x}K. In this wash a considerable fraction, but not all, of the extracellular ^{x}K would be lost, according to the time scale of its removal found on p. 280. The uptake curves (Fig. 3) do not, for this reason, show so rapid an increase of ^{x}K in the first few minutes as would correspond to the loss curves of Fig. 2, but they do include 0·3–0·6 μequiv ^{x}K (depending on muscle size) left in the extracellular space, and this is one factor to be allowed for in making the comparison.

At 0° C the uptake of ^{x}K, although initially rapid, appears to approach a value less than the value approached by the respective pair muscles in the same solution at 18° C. An additional rapid uptake took place if the temperature was raised, as is shown by one of the curves. This is true for all the runs made at 0° C, and holds even after 20 hr exposure to the ^{x}K solution. It is difficult to assess the accuracy of the procedure, because reproducibility of washing and muscle geometry beneath the counter sets the limitation, but readings of the ^{x}K content should be correct to about 5%, judged from the scatter when the content is changing but little. There can be no question that behaviour at 0° C differs from that at 18° C, just as was the case in the soaking-out experiments described on p. 281. It must be remarked that some warming up of the 0° C specimens took place during each assay, and this will tend to reduce the observed temperature effect. It is not suggested that at 0° C uptake of ^{x}K ceases before complete exchange is reached, but results indicate that the amount entering per hour drops eventually to a value equal to about 2% of the total internal potassium.

There are a number of reasons why quantitative agreement between the degree of equilibration of muscle potassium and external potassium found

by the two procedures (observation of ^{x}K entry, or exit, respectively) should not be exact. The more important are (*a*) presence of some ^{x}K in the extracellular spaces after the short wash given in the ^{x}K entry method; (*b*) the result of the succession of re- and de-polarizations of the phase boundary brought about by exposure to high-potassium solution and low-potassium wash fluid, which may well induce a more rapid turnover of the ions; and (*c*) the fact that entry of ^{x}K into *fresh* normal muscles was followed; whereas

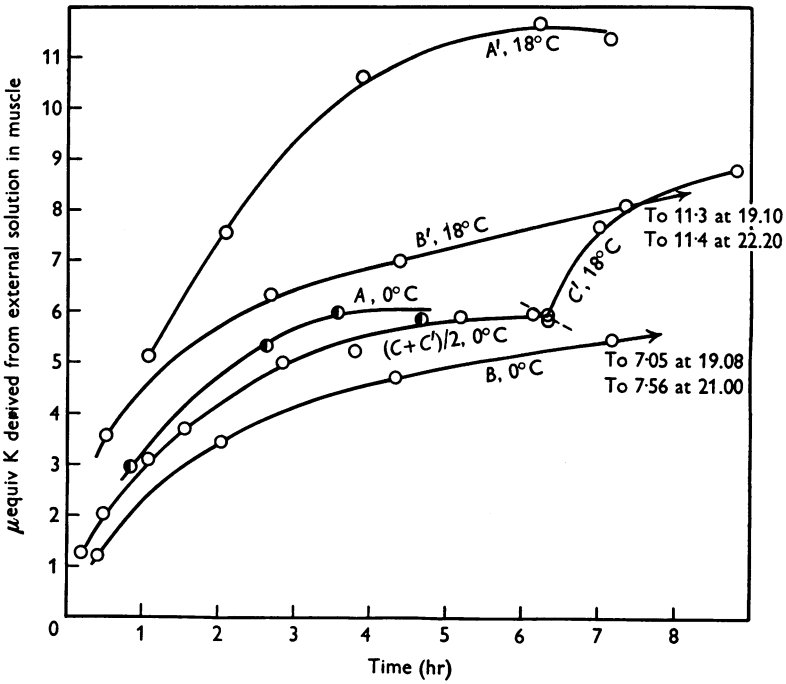

Fig. 3. The uptake of ^{x}K from ^{x}K phosphate at 0 or 18° C by sartorius muscles. The muscles have connective tissue, and at the pelvic end, a fragment of bone, attached. These substances also take up ^{x}K, but when being assayed they are so situated with respect to the counter that they do not contribute more than 10 % to the total count rate as was found at the end of the experiment by cutting out the intervening muscle. The ^{x}K contents of the muscles only at the end of the run are given in Table 1. Runs *A*, *A'*; *B*, *B'* are made on the individuals of pairs; run *C* was made on two muscles together for the first period (6 hr 20 min), one was removed for analysis and the run was continued at 18° C. In order to make the ^{x}K content comparable during the first phase the amount plotted is (^{x}K in two muscles + connective tissue)/2.

to prepare muscles containing ^{x}K for efflux experiments a prior exposure to a high-potassium medium has been given, whether in the plasma of the injected frog (potassium as high as 8 m.equiv/l.) or in ^{x}K phosphate. Thus the prepared muscles have gained potassium during pretreatment, and do not suffer so drastic a change of environment as do the fresh muscles, which can promptly

gain a proportion of ^{x}K without exchanging potassium for it; they may, for example, lose sodium instead.

In view of consideration (*c*) it was thought useful to try some runs, using ^{x}K in the solution, but starting with muscles which had already been soaked in ordinary potassium phosphate first. Fig. 4 illustrates the results of these runs. In view of the variable behaviour of the untreated muscles (Fig. 3) the effect would have to be gross to be significant. The treated muscles

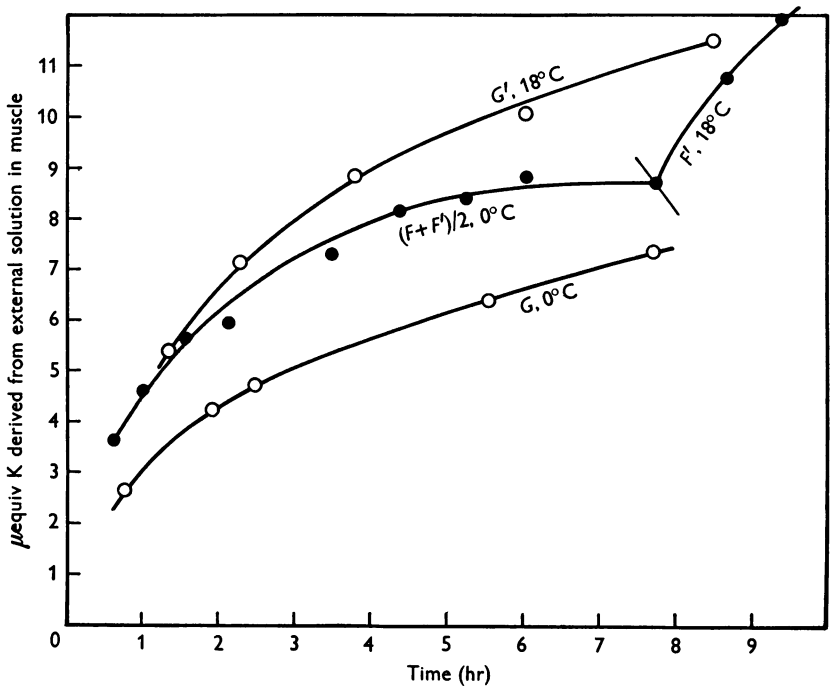

Fig. 4. The uptake of ^{x}K from ^{x}K phosphate by muscles which had first been exposed to ordinary potassium phosphate solution (for details see the latter part of Table 1). The first phase of one run, marked $(F+F')/2$, refers to uptake by two muscles plus connective tissue, divided by two to make results comparable with those for single muscles. The later phase of the same experiment, carried out at 18° C, refers to one only of the muscles plus connective tissue.

approach equilibrium as, or more, slowly than the slower ones (*B*, *B'*) of Fig. 3, and at 18° C specimens fail to reach as high a ratio $^{x}K/K$ as the untreated ones in similar or shorter times, as will be seen in Table 1 (compare *F'*, *G'*, *H'* with *A'*–*E'*). The reduced equilibration of *C'* may also be due to its prior exposure at 0°. The table summarizes the end results of a number of experiments; it will be noticed that the K content of the muscles exceeds 100 μequiv/g. The mean content of fresh muscles is less than this; $93{\cdot}5 \pm 9{\cdot}9$ μequiv/g (S.E., thirty-nine analyses), so potassium has been gained

TABLE 1. The degree of equilibration of tissue potassium with labelled potassium derived from the phosphate solution (140 m.equiv K/l.)

Muscles freshly dissected

Ref. to Fig.	Duration of exposure to ^{z}K phosphate hr	min	Final total potassium content (μequiv)	Ratio: ^{z}K/K	Mass muscle + ends: Initial (mg)	Mass muscle + ends: Final (mg)	Muscle only (mg)
^{z}K soak at 0° C:							
A (Fig. 3)	5	10	13·0	0·45	154	132	Not weighed
B (Fig. 3)	21	0	8·8	0·80	132	105	84
C (Fig. 3)	6	20	7·25	0·79	See *C′*		71
D —	18	32	10·7	0·73	Not weighed		94
E —	3	24	6·6	0·59	58	56	56
^{z}K soak at 18° C:							
A′ (Fig. 3)	7	7	11·0	0·99	141	133	108
B′ (Fig. 3)	22	20	11·0	0·95	142	137	106
C′ (Fig. 3)	6 + 2	20 at 0° + 45 at 18°	8·65	0·90	(*C* + *C′* 158	167)	71
E′ —	3	3	6·4	0·93	56	54	54

Muscles pre-treated in potassium phosphate solution

Pre-treatment: Duration (hr)	Pre-treatment: Temperature (° C)	Ref. to Fig.	Duration of exposure to ^{z}K phosphate hr	min	Final total potassium content (μequiv)	Ratio: ^{z}K/K	Mass muscle + ends: Initial (mg)	Mass muscle + ends: Final (mg)	Muscle only (mg)
		^{z}K soak at 0° C:							
16	0	*F* (Fig. 4)	7	42	11·25	0·79	See *F′*		102
2	0	*G* (Fig. 4)	7	43	11·2	0·61	133	137	106
2	0	*H* —	3	15	10·3	0·49	99	104	104
		^{z}K soak at 18° C:							
16	0	*F′* (Fig. 4)	7 + 1	42 at 0° + 40 at 18°	13·1	0·83	(*F* + *F′* 291	250)	122
2	18	*G′* (Fig. 4)	8	26	14·3	0·73	135	158	121
2	18	*H′* —	2	39	9·8	0·71	95	98	98

Notes. The K content, and ratio ^{z}K/K, refer to the muscle without the ends, *except* Expt. *A*. The individuals of pairs are shown by *A*, *A′*, etc.

in the experiments. The mass of the specimens underwent erratic variations upon exposure to the phosphate solution, and it is for this reason that it is impracticable to compare uptakes per unit mass of tissue.

DISCUSSION

The results show that at 18° C all, to within say, 5%, of the potassium of frog muscle will exchange with external potassium in a simple exponential manner. At 0° C, 20% of the potassium is less readily exchanged than is the rest. Accordingly the influence of temperature is twofold, it causes mobilization of internal potassium, and affects the rate of transfer across the cell boundary.

The possibility of some muscle potassium being bound was indicated by Brues, Wesson & Cohn (1946), and both Mullins (1942) and Szent-Györgyi (1945) claim to have shown specific combination between potassium and the principal protein of muscle, myosin. If some potassium is bound in the cell there should be an indication of it in the ratio of the ionic activities according to the equation

$$E = \frac{kT}{\epsilon} \ln \frac{f_i[\mathrm{K}_i]}{f_e[\mathrm{K}_e]},$$

the f's being activity factors. Recent measurements of the potential (Nastuk & Hodgkin, 1950; Ling & Gerard, 1949) agree in finding E about 100 mV at 20° C and $[\mathrm{K}_e] = 2$ m.equiv/l. With $[\mathrm{K}_i] = 130$–140 m.equiv/kg fibre water, and equal internal and external activity factors, the calculated figure is only 5 to 7 mV higher, which may be within experimental error, but which could equally indicate that the activity of the internal potassium is somewhat reduced, because the logarithm makes the value of E insensitive to the ratio once the latter is high. The effect of temperature on E, investigated by Ling & Woodbury (1949) is relevant. These authors found $E = 71$ and 83 mV at 5° and 23° C respectively in a solution with $[\mathrm{K}_e] = 5$ m.equiv/l. The change of E is greater than would be caused by the change in T in the equation. An explanation for the increased effect of temperature is provided if f_i changes with temperature, as the exchangeability results suggest. If one takes $f_i[\mathrm{K}_i] = 140$ m.equiv/kg fibre water at 23°, and only 110 m.equiv/kg at 5° the calculated E's come to 74 and 85 mV respectively at 5° and 23° C.

There are several possible explanations of failure to maintain a high rate of turnover between internal and external potassium at 0° C. (*a*) An essential metabolite may be present in limited amount, and not be re-formed at 0° C, so that once it is exhausted the rate of turnover diminishes; or (*b*) some of the potassium may be combined as a slightly ionized compound so that equilibration depends upon the rate of (ionized) $\rightleftharpoons$ (un-ionized) transformation; or (*c*) some potassium may be confined in a phase only accessible by mediation

of a process which is very slow at 0° C. The latter possibility includes that of there being a proportion of the cells making up the muscle differing in some way, at 0° C, from the rest. The experiments in which muscles were pre-treated at 0° C do not support suggestion (*a*); indeed it seems possible that it is pre-treatment at 18° C which leads to exhaustion.

It does not, therefore, seem possible to draw any final conclusion about the reason for the low turnover rate of some of the muscle potassium at 0° C.

SUMMARY

1. The exchange of the potassium of frog muscle has been investigated in potassium phosphate media using radio-potassium preparations.

2. To saline solution, a rapid exit of the quantity of labelled potassium expected in 0·19 of the muscle volume occurs.

3. In potassium phosphate, at 18° C, following rapid turnover of 20–30% of the potassium, the remainder follows first-order kinetics with rate constant about 0·5 hr^{-1}.

4. In potassium phosphate at 0° C the whole equilibration process is slower, and about 20% of the potassium appears to exchange only very slowly.

5. The possibility of the activity coefficient of the internal potassium being reduced at 0° C is discussed in relation to the temperature coefficient of the membrane potential.

My thanks are due to Prof. A. V. Hill for his continued interest in, and support of, this work. Some of the expenses were covered by a grant from the Government Grants Committee of the Royal Society.

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