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THE EXCHANGE OF FROG MUSCLE POTASSIUM

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The rate of incorporation of potassium into isolated frog sartorii was measured by Harris & Burn (1949). Radio-potassium was employed as tracer. The results were scattered, partly because a number of different temperatures were used, and partly as a consequence of the net loss of potassium which usually took place. The muscles were not in a steady state. By substituting a mixed chloride + bicarbonate medium for the chloride solution previously employed it has proved possible to keep some muscles for as long as 24 hr at 18° C without appreciable loss of potassium. Further tracer experiments were therefore made under the improved conditions to examine the effect of temperature and concentration on the rate of potassium exchange.

In principle, the potassium turn-over can be observed either by following the incorporation of radio-potassium from a solution, or by pre-treating the muscle so that it contains some tracer and then following the loss of radioactivity to a tracer-free solution. Both methods have been used. The results indicate that the properties of the total intracellular potassium cannot be derived from the observations of the tracer ions on the assumption that there is rapid and complete mixing of the tracer ions with the whole of the muscle potassium. For example, in an 8 hr experiment less than half of the potassium behaved as if it were freely exchangeable. This observation is in qualitative agreement with earlier results obtained in isotonic potassium phosphate at 0° C (Harris, 1952). An explanation would be provided if some of the potassium is bound to the muscle substance in the form of a slightly ionized compound.

METHODS

Storage. Experiments were made using muscles of *Rana temporaria*, usually the sartorius, semimembranosus and semitendinosus. Muscles were either analysed directly or after 24 hr storage in a mixture of the following composition (in m.mole/l.): K 3, Na 126, Ca 2, Mg 1, SO_4 1,

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Cl 97, phosphate 3, bicarbonate 30. In some experiments the sodium salt of a specified acid was also added in concentration 5 m.mole/l. Oxygen with 5% CO_2 was bubbled into the mixture at 18° C; the pH was 7·2.

Kinetic experiments. All experiments were made on the sartorii of *R. temporaria*. These were carefully dissected and a fragment of bone was left attached at the pelvic end. The solutions used for 2–8 m.equiv/l. K concentrations were made by mixing Na_2CO_3 with the correct proportions of HCl and salts to provide K 2–8 m.equiv/l., Cl 94 plus number of m.equiv K, other ions as specified above. For experiments at 18° C oxygen with 5% CO_2 was introduced, for 0° C experiments 1·5% CO_2 in oxygen was used (pH 7·4). For higher K concentrations the solution employed was made up from potassium and sodium phosphates, 0·1 M with respect to phosphate, the proportions of mono- and di-basic salts being adjusted to give pH 7.

The radioactive potassium preparations were made from irradiated K_2CO_3 obtained from A.E.R.E. Harwell. For certain experiments possible contamination of the K with Na was reduced by precipitation of $KClO_4$. The perchlorate was converted to chloride by heating. To carry out an experiment the muscles were provided with cotton loops at each end so that they could be stretched between two pegs mounted on a Perspex base. When so held between the pegs they could be slid beneath an end-window Geiger tube for assay of their radioactivity. The muscles were exposed for a time to a radioactive solution, freely drifting about in the fluid into which the gas mixture was bubbled. During this exposure assays of muscle radioactivity were made at intervals. For this the muscle was withdrawn and before putting on the frame, washed for either exactly 1 min or 15 sec in non-radioactive solution of the same composition and at the same temperature in order to remove adhering radioactive fluid. The radioactivity was measured for 2 min and then the muscle was returned to the solution. After about 4 hr exposure to the radioactive solution the specimen was transferred to a non-radioactive solution, otherwise similar, and loss of radioactivity observed. During this phase of the experiment it was not necessary to wash before assaying the radioactivity.

At the end of about 4 hr the specimen was removed, assayed as usual, and then dissolved in acid. An aliquot of the muscle solution was counted and its activity compared with that of a known dilution of the radioactive soaking solution used during the first phase of the experiment.

The volume of soaking solution used in all experiments was about 50 ml. This volume contains 10 or more times (depending upon the K concentration) the total K of the muscle. As exchange between muscle and solution was far from complete it would not be too inaccurate to regard the specific radioactivity of the solution K as unchanged during the soaking of the muscle, but as a precaution the solution was renewed at least once.

Readings of radioactivity were corrected as necessary for blank, resolution time and decay. Potassium analyses were carried out by the cobaltinitrite method; at the levels encountered (5–10 μequiv) the standard error of determinations was 0·2 μequiv (i.e. about 3%).

RESULTS

Storage trials

Fenn & Cobb (1934) examined the retention of K by sartorii stored in bicarbonate media. They remark that while the various muscles of a single frog differ so much that one does not provide a useful control for the others, the individuals of a pair did usually, but not always, have nearly equal K contents. A difficulty in assessing the results of storage experiments is the fact that any slight damage, bacterial infection, or deficiency of metabolite in the frog (at the time of dissection), may lead to loss of K without the reason being obvious. Thus it is not particularly useful to obtain averages for the K contents before

and after storage, because some storage results have to be rejected for unknown causes. It seems most important to show that *some* muscles can be kept for 24 hr at 18° C in the solution without serious loss of K (Table 1). Potential metabolites were added to the solution for some of the trials but the scatter of the results does not permit any evaluation of their relative merits; they certainly

TABLE 1. Potassium contents of muscles after 24 hr storage at 18° C.

Solution used	K contents, μequiv/g
(1) Bicarbonate + chloride with 3 m.equiv/l. K	76, 77, 79, 82, 93, 93, 103, 111, 118, 81, 101, 86, 81
(2) As (1) with addition of 5 m.mole/l. Na ketoglutarate	71, 82, 91, 91, 92, 96
(3) As (1) with addition of 5 m.mole/l. Na glutamate	74, 79, 87, 90, 96, 80, 81
(4) As (1) with addition of 5 m.mole/l. Na cysteine	71, 102, 104, 119
(5) As (1) with addition of 5 m.mole/l. Na ascorbate	72, 87, 87, 89, 92

Fresh sartorius muscles taken from the frogs contained 94±12 (S.D.) μequiv K/g.

do not bring about any noticeable improvement in comparison with the bicarbonate solution alone. The only compound tested which had a drastic influence was thiolacetic acid ($CH_3.COSH$) which induced rapid K loss. The muscles were tested qualitatively for excitability; this was always retained in the specimens to which the figures below refer. Some measurements of tetanus tension were made by Mr B. C. Abbott before and after storage. After storage the figure found was 85–100% of the original value.

Uptake and loss of radio-potassium in vitro

A series of experiments was made in the following way. A muscle was exposed for about 4 hr to a solution containing a proportion of radio-potassium (denoted *K) so that the degree of replacement of the original K by that derived from the solution could be found. A number of readings of activity, giving a measure of the K exchanged, were made during the period. The specimen was then put into a solution identical chemically, and at the same temperature, but free from radioactive tracer. The loss of radioactivity from the muscle was observed for a further period of about 4 hr. Fig. 1 illustrates some runs. Uptake is rapid for about 20 min and then either becomes nearly linear, or has throughout a diminishing rate. Empirically it is possible to fit the uptake curves by means of the equation

$$*K = A\,(1-\exp(-at)) + B\,(1-\exp(-bt)).$$

The time course of the loss of radioactivity after transfer to an inactive solution at time t_1 should be given by

$$*K = A\,(1-\exp[-at]) + B\,(1-\exp[-bt]) - A\,(1-\exp[-a\,(t-t_1)]) - B\,(1-\exp[-b\,(t-t_1)]),$$

according to the superposition theorem, which will hold provided a steady state pertains. I am indebted to Mr A. F. Huxley for pointing out to me this relation between the two phases. In nearly all runs a good fit of the complete curve can be obtained by use of the equations, and it was only in those cases in which there was reason to suspect K loss during the experiment that a given set of constants could not be found to describe the whole curve.

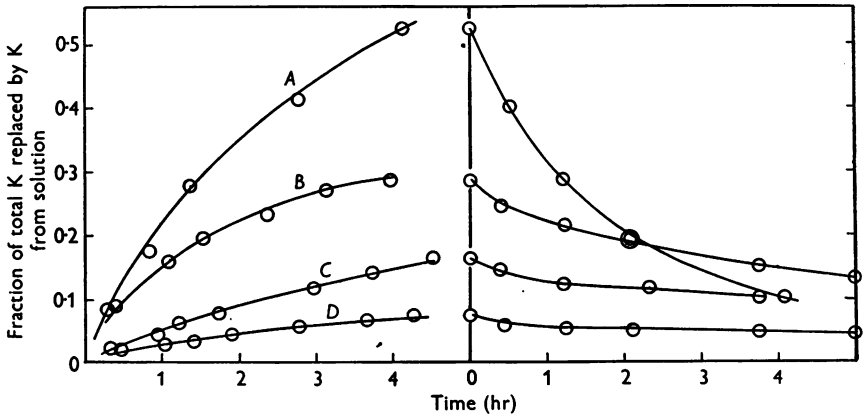

Fig. 1. The time course of the uptake of labelled K from solutions of stated K concentration at different temperatures (rising phase), and (falling phase) the loss of the labelled K to solutions of the same composition and temperature but having ordinary K instead of labelled K. Before each assay of radioactivity during the rising phase the muscles were given a 15 sec wash. *A*, 8 m.equiv K/l. 18° C; *B*, 8 m.equiv/l. 0° C; *C*, 2 m.equiv/l. 18° C; *D*, 2 m.equiv/l. 0° C.

The constants can be evaluated from the experimental curves. *A* proves to be 1–5% of the total muscle K; it is 2–3 times the calculated extracellular K. In K concentrations of 2–8 m.equiv/l. the value of a is about 3 hr^{-1}, insufficient observations being available to permit accurate evaluation. It is possible that a is higher (corresponding to more rapid equilibration) in the stronger K solutions. The quantity *B* represents a part of the muscle K which exchanges comparatively slowly, the rate constant being 0·06–0·4 hr^{-1}. The peculiar feature is that in media having low K concentration *B* is much less than the total K content of the muscle. It is as if a considerable part of the muscle K does not mix with the parts *A* and *B* undergoing exchange. Tables 2 and 3 summarize the results of a number of experiments. It appears that increase of temperature may cause either *B* or b to rise. Increase of external K increases the rate of replacement, both *B* and b being influenced. Some calculations were made to see how well the curves could be fitted by various pairs of values of b and *B*. It proved possible to obtain as good a fit over a range of *B* covered by the factor 1·15, provided b was shifted at the same time in the other direction so that the product was nearly constant. That is to say, any value given for b or *B* might be 15% too high or too low.

The net rate of uptake of labelled material during the first phase of the experiments has also been included in Tables 2 and 3. The interval between 20 min and 1 hr 20 min was chosen for measurement of this figure because after 20 min the initial rapid turn-over is nearly complete. These rates, and particularly the effect of temperature upon them, are more consistent than the separate values of b and B.

TABLE 2. Rates of incorporation of K as measured by the gain of labelled K in the interval 20 min to 1 hr 20 min and values of the parameters in the equation for uptake: $*K = A(1-\exp(-3t)) + B(1-\exp(-bt))$, and in the corresponding equation, given in the text, for loss of *K to an unlabelled solution. All results refer to pair muscles, one being soaked at 0° C and the other at 18° C

Ext. K concn. (m.equiv/l.)	Ref. to Fig.	Muscle mass (mg)		Final K content (μequiv/g)		Fraction of K replaced in 1 hr interval		Ratio 18°/0° C	Value (as % of total K) of A		B		b (hr^{-1})	
		0°	18°	0°	18°	0°	18°		0°	18°	0°	18°	0°	18°
2	1 *D*, 1*C*	62	67	90	82	0·016	0·040	2·5	1·5	1	22	43	0·06	0·095
2	—	94	108	97	88	0·013	0·024	1·0	1	1·5	24	33	0·077	0·084
2	—	36	35	92	88	0·013	0·044	3·4	1	1	17	43	0·08	0·12
4	2 *B*, 2 *A*	60	61	100	90	0·041	0·089	2·2	1	1·5	61	71	0·06	0·145
4	—	45	47	90	93	0·042	0·075	1·8	1	1	34	57	0·13	0·16
4	—	129	117	86	90	0·021	0·052	2·5	1	1	44	40	0·045	0·13
8	1 *B*, 1 *A*	32	35	89	82	0·085	0·156	1·8	3	3	61	62	0·14	0·39
8	—	56	48	112	94	0·035	0·10	1·9	3	3	36	56	0·11	0·19
8	—	71	72	90	90	0·067	0·14	2·1	3	3	43	61	0·17	0·24
8	—	63	64	108	81	0·062	0·15	2·4	1·5	2·6	67	89	0·086	0·186
18	—	116	117	92	91	0·063	0·11	1·8	1	3	39	83	0·19	0·16
18	—	110	120	92	104	0·059	0·09	1·6	1	1	36	55	0·16	0·18
18	—	70	68	113	109	0·048	0·115	2·4	1·5	3	40	40	0·12	0·43
37	—	66	68	86	82	0·10	0·20	2·0	5	4	43	96	0·23	0·24

TABLE 3. Rates of incorporation of K as measured by the gain of labelled K in the interval 20 min to 1 hr 20 min and values of the parameters in the equation for uptake: $*K = A(1-\exp(-3t)) + B(1-\exp(-bt))$, and in the corresponding equation, given in the text, for loss of *K to an unlabelled solution. Results for pair muscles in dissimilar solutions at equal temperatures

Ext. K (m.equiv/l.)...	Muscle mass (mg)		Final K content (μequiv/g)		Fraction of K replaced in 1 hr interval		Ratio $\frac{4\text{ m.equiv}}{2\text{ m.equiv}}$	Value (as % total K) of A		B		b (hr^{-1})	
	2	4	2	4	2	4		2	4	2	4	2	4
Temp:													
0° C	47	50	79	79	0·016	0·041	2·5	1	1	25	45	0·65	0·095
18° C	44	44	89	100	0·049	0·091	1·85	1	1·5	42	62	0·14	0·17

Stretch

Feng (1932) showed that the resting metabolism of muscle is increased by application of tension. It was thought that this might be reflected in an increased rate of K turn-over. During four runs, made otherwise as before, the muscles were tied between two hooks during an interval so that their lengths were increased by about 10%. Fig. 2 illustrates two such runs: no perceptible increase of the rate of K turn-over was induced.

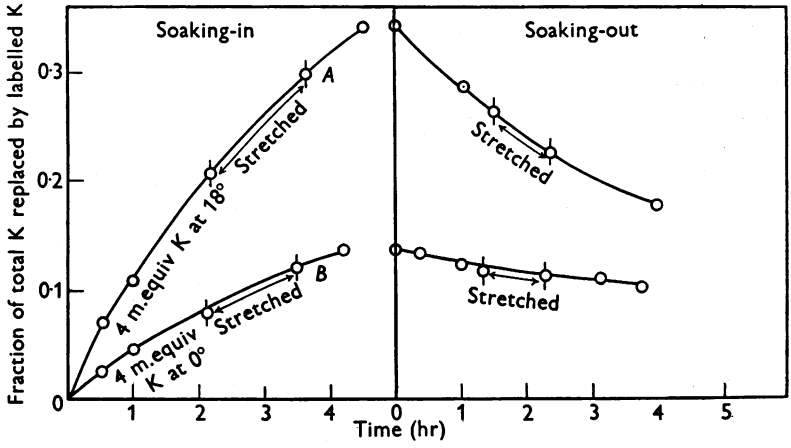

Fig. 2. Uptake and loss of radioactivity by muscles which were stretched between the times indicated. Stretch seems to be without influence. The solution had 4 m.equiv K/l.

DISCUSSION

The storage experiments were carried out primarily to find a solution which could be used for the exchange experiments without there being a simultaneous net loss of K. Since muscles stored in chloride media for 24 hr at 18° C were found to lose 20% or more of their K (Harris & Burn, unpublished results) it seems that the bicarbonate medium is superior. Creese (1952) has reported that bicarbonate ion favours the retention of K by rat muscle, in which tissue the same author found all the K to be exchangeable (1951).

The high K content of the fresh muscles is worthy of remark. 90 μequiv/g corresponds to an internal concentration of about 140 μequiv/g water, which is higher than the commonly accepted figure (approx. 120 μequiv/g water), taking the fibre water as 64% of the muscle weight.

The results of the tracer experiments indicate that in the physiological range of K concentrations not all of the muscle K is freely exchanged *in vitro*. The fast process, involving a few per cent of the total K, may well be explained on the basis of the diffusion of K into some damaged fibres; the equilibration of the extracellular space alone would not suffice to account for the quantity of K involved. The rate of the slower exchange, involving a fair proportion of the muscle K, as shown in Table 2, can be expressed in terms of the fraction of the total K replaced per hour. In a solution containing 2 m.equiv/l. about 1·5% at 0° C, and 4% at 18° C is so replaced; the proportions increase as external K is raised, and in the 37 m.equiv/l. solution the respective figures are 10 and 20%. When, however, not all the K is taking part in the turn-over the content of labelled material which can be introduced approaches an asymptote lower than the total K content found by analysis.

If only uptake experiments had been made, instead of following both uptake and loss of the labelled material, it would have been much more difficult to detect the failure of some of K to participate in the turn-over; this would only be shown by a curvature of the plot of labelled K content versus time towards an asymptote lower than the total K content. When, however, the loss of the labelled material is observed it proves that, *if* the measured rate constant applies to all the K, a considerable unbalance between influx and efflux must exist. This would be sufficient to cause, for example, the loss of as much as half the total K in an experiment lasting 8 hr. As the final analyses show that any loss must have been much less than this it is clear that it is incorrect to assume that all the K is equally readily exchangeable under the conditions used. That is, the rate constant found for tracer movement must not be applied to the whole of the K.

The conclusion that the K does not reach full exchange in the saline solution is supported by the results of experiments in which the muscles were exposed for longer times to the radioactive saline. For example, after both 16 and 18 hr at 18° C in saline having 4 m.equiv/l. K, the exchange attained was 50% in each of two muscles and no further increase of radioactivity was taking place. One muscle was used for K analysis and the other was treated for a further 1·7 hr with radioactive potassium phosphate solution of specific activity equal to that of the K in the saline mixture. The extracellular phosphate was next removed by returning the muscle to radioactive saline for 1 hr and the radioactivity and K content were returned. The degree of exchange was now 74% and the K content, 99 μequiv/g, was equal to that of the other muscle. That is to say, the treatment in potassium phosphate has considerably increased the degree of exchange without increasing the K content of the muscle. The increase brought about corresponds fairly well to the amount expected on the basis of the measurements of rate of exchange in potassium phosphate solution made previously (Harris, 1952).

It may be difficult to accept the conclusion that in media having low K concentrations only 40% at 18° C of the muscle K is freely exchangeable, and even less at 0° C. A physical explanation, based on the very slow diffusion of some of the K, has to be kept in mind, but no support is given to this by the fact that the smaller muscles do not behave differently from the larger ones. In Table 2, the muscle masses differ by a factor of up to 4. A comparison made by Abbott (1952) showed that the rate of exchange of the K of the toe muscle (weighing only about 3 mg) does not greatly differ from the rate found to apply to the sartorius muscle of the same frog. Thus mere bulk of the muscle up to the size of the sartorius does not appear seriously to hinder K movements.

Returning to the possibility of chemical combination, it is to be noted that (*a*) it is reported that the resting potential does not continue to rise as external K is reduced as much as would be expected, and the magnitude of the resting

potential in solutions having low K concentration only corresponds to an inside/outside concentration ratio of about half that found by analysis, and (*b*) the temperature coefficient of the resting potential is higher than it would be if the concentration ratio were unaffected by temperature. There are, of course, alternative explanations of these facts, but it does appear that there is good reason to suspect formation of an un-ionized K compound.

SUMMARY

1. The rates of uptake and loss of tracer-labelled potassium by isolated frog sartorii have been measured under various conditions.

2. Storage experiments were made to show that use of a mixed chloride-bicarbonate medium permits isolated muscle to be kept for 24 hr without serious loss of potassium.

3. The kinetics of uptake and loss of tracer potassium can be described in terms of two first-order processes. One has a time constant of about 20 min and involves 1–5% of the total potassium, and the other has a time constant of 2–16 hr and involves 20–100% of the total potassium. In all except one experiment a part of the potassium did not appear to undergo exchange.

4. Increasing temperature from 0 to 18° C in some cases increased the rate constant of the turnover of the slower fraction and in other cases it increased the proportion of potassium participating in the turnover.

5. Increasing the external potassium concentration tends to increase the proportion of exchangeable potassium at 18° C.

6. Attention is drawn to an error which can arise if tracer observations without regard to analyses are used to determine the efflux.

My thanks are due to Dr P. C. Caldwell for permission to use some results of storage experiments, and to Mr B. C. Abbott for help with some of the kinetic experiments. Part of the expenses of the work was met by the Government Grants Fund of the Royal Society.

REFERENCES

Abbott, B. C. (1952). Potassium exchange in frog muscle. *J. Physiol.* **117**, 24 *P*.

Creese, R. (1951). Exchangeability of muscle potassium. *J. Physiol.* **115**, 23 *P*.

Creese, R. (1952). Bicarbonate ion and muscle potassium. *Biochem. J.* **50**, xviii.

Feng, T. P. (1932). The effect of length on the resting metabolism of muscle. *J. Physiol.* **74**, 441–454.

Fenn, W. O. & Cobb, D. (1934). The potassium equilibrium in muscle. *J. gen. Physiol.* **17**, 629–656.

Harris, E. J. (1952). The exchangeability of frog muscle potassium studied in phosphate media. *J. Physiol.* **117**, 278–288.

Harris, E. J. & Burn, G. P. (1949). The transfer of sodium and potassium ions between muscle and the surrounding medium. *Trans. Faraday Soc.* **45**, 508–528.