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THE EXTRACTION OF IONS FROM MUSCLE BY WATER AND SUGAR SOLUTIONS WITH A STUDY OF THE DEGREE OF EXCHANGE WITH TRACER OF THE SODIUM AND POTASSIUM IN THE EXTRACTS

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Tracer studies reveal that part of the K (Harris, 1952, 1953) and part of the Na (Conway & Carey, 1955) of frog muscle do not equilibrate with the respective tracer applied to the outside. The question arises: what is the site of the unexchanged part in each case? It may happen that there are two distinct fractions present, one which exchanges fully, and the other relatively inexchangeable, or there may be a graduated ease of exchange as would arise, for example, if the ions were held on sites of differing energies of absorption or different distances from the periphery. The observation of Tobias (1950) that a small fraction of the Na cannot be leached out of muscle by prolonged soaking in distilled water suggests some firm binding for part of the Na.

In the present paper the specific radioactivity of the Na or K has been determined in successive extracts from muscles previously soaked in the respective tracer. The inextractable Na proves to acquire little radioactivity. The K of muscle and nerve proves to undergo a non-uniform degree of exchange with the tracer so that the specific activity diminishes in successive extracts. Furthermore, exchange of the final fractions is far from complete even after long pretreatment with tracer.

A possible explanation for the behaviour of the K can be provided if movement of this ion takes place through long pores or in a matrix of fixed charges. In these conditions the rate of tracer movement against a net outward movement can become zero before full exchange has been reached.

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METHODS

The smaller skeletal muscles of the hindlimb of *Rana temporaria* were used. These usually included the sartorius, biceps, semitendinosus and peroneus. To determine tracer ion uptake in the exchange experiments the muscles were immersed for various times in a saline mixture containing 90 mM-NaCl, 30 mM-$NaHCO_3$, 1 mM each $MgCl_2$ and $CaCl_2$, and KCl 2 mM or as stated. This was bubbled with 95% O_2 + 5% CO_2, giving a pH of about 7·4. According to the exchange being studied either the Na or the K salt was prepared from an irradiated sample of spectroscopically pure carbonate. After immersion the muscles were prepared for analysis and radioactivity assay either by wet combustion in nitric acid, or by dry combustion at 550°.

For the study of the extraction of ions by water or sugar solution the muscles were first loaded by immersion in saline containing either tracer Na or K, and then they were immersed for a number of periods in successive portions of the extraction medium. The portions were then used for radioactivity assay, and analyses were made for Na and K, and sometimes for phosphate and Cl. The sugar used was sometimes sucrose, sometimes glucose; results were similar.

Alkali analyses were made using a flame photometer. Phosphate was determined by the Berenblum–Chain method, and Cl by the method of Schales & Schales (1941).

RESULTS

Sodium

That not all the muscle Na will equilibrate with labelled Na in 4 hr at room temperature or in 16–20 hr at 4° is shown in Table 1. Usually between 4 and 8 m-equiv/kg (average 5 m-equiv/kg) fails to exchange. Treatments which lead to increase of muscle Na do not noticeably alter the amount which remains unexchanged.

The homogeneity of the muscle Na was examined as follows. The specific activity (s.a. = ratio radioactivity/content of Na) of the Na in the successive aqueous extracts of loaded muscles was measured. The results in Table 2 were obtained using about 1 g muscle (made up of five small muscles) which was passed through 50 or 25 ml. portions of water. Most (about 80%) of the

TABLE 1. Equilibration of muscle Na with tracer

Treatment	Na content by analysis (m-equiv/kg tissue)	Tracer Na content (m-equiv/kg tissue)	Difference (m-equiv/kg tissue)
Soaked 4 hr at 18–20° C	43·9	40·2	3·7
	44·3	37·6	6·7
	31·4	27·1	4·3
	49·1	45·5	3·6
	38·5	37·1	1·4
Soaked overnight at 4° C	54·1	49·6	4·5
	49·6	48·9	0·7
	45·5	39·5	6·0
	41·6	37·2	4·4
	41·6	33·5	8·1
	35·2	30·3	4·9
Soaked overnight at 4° C in 150 mM-Na	82·0	72·0	10·0
Soaked overnight at 4° C with 10 μg/ml. strophanthin	107·0	101·5	5·5

muscle Na is extracted within 1 hr. The specific activity of this part is high, close to that of the pretreatment solution. The specific activity of the Na in later extracts falls. The value is however particularly low in the Na associated with the residual water-swollen tissue. This residue contains less than 5 % of the total Na of the normal muscle (cf. Harris & Steinbach, 1956, expt. 1 *a*). The same procedure was repeated, but at the conclusion the muscle residue was divided into three, viz. the outer quarters (ends) and the middle. This showed that the ends contained about three times as much Na as the middle, while the specific activity of the Na in the ends was less than one-third of that

TABLE 2. Specific activities and quantities of K and Na leached from muscles by serial extraction in water

Expt.	Solution	Specific activity (counts/min/μequiv)	Quantity (μequiv/g tissue)
	Na experiments		
1	Pretreatment in tracer Na, 5 hr	Not measured	—
	Extract, 0–1 hr	47,900	38·0
	Extract, 1–10 hr	37,400	8·0
	Tissue		
	Terminal 1/4's	3,460	3·47
	Middle	11,500	1·13
2*a*	Pretreatment in tracer Na, 4 hr	31,000	—
	Extraction of terminal 1/10's		
	Extract, 0–1 hr	31,000	40·8
	Extract, 1–24 hr	31,800	6·8
	Extract, 24–25 hr	300	1·3
	Residue (ends)	16	6·5
2*b*	Pretreatment in tracer Na	31,000	—
	Extraction of middle part of muscles		
	Extract, 0–1 hr	31,000	22·0
	Extract, 1–24 hr	29,700	7·0
	Extract, 24–25 hr	500	2·0
	Residue (middle)	314	2·2
	K experiments		
3	Pretreatment in tracer K, 4 mM, 16 hr, 14° C	1,690	—
	Extract, 0–20 min	1,150	5·2
	Extract, 20–70 min	840	12·9
	Extract, 70–280 min	680	29·4
	Residue		
	Terminal 1/4's	630	3·2
	Middle	500, 696	4·0
4	Pretreatment in tracer K, 14 mM, 16 hr, 14° C	1,990	—
	Extract, 0–20 min	1,550	8·5
	Extract, 20–70 min	1,400	13·5
	Extract, 70–280 min	1,160	29·5
	Residue		
	Terminal 1/4's	1,137	3·5
	Middle	1,280, 1,086	5·2

Swelling of ends and middle assumed equal. Average swelling, at end of extraction, was to 1·2 × original weight.

of the Na in the middle part (Table 2, Expt. 1). Another trial in which the terminal tenths of the muscle were compared with the intermediate 8/10 showed an even greater difference of specific activity; the ends retained about three times as much Na as the middle per unit weight, but the specific activity of the Na in the ends was so low as to be measurable only with difficulty, while that of the Na in the middle section was appreciable (Table 2, Expt. 2).

These results strongly suggest that a small fraction of the muscle Na is nearly inexchangeable and that it resists water extraction. It is associated with the ends of the muscle and therefore likely to be held in the connective tissue. Such connective tissue must differ from true tendon, for a trial with a piece of Achilles tendon showed that this did not retain inexchangeable Na more than did the middle part of the muscle.

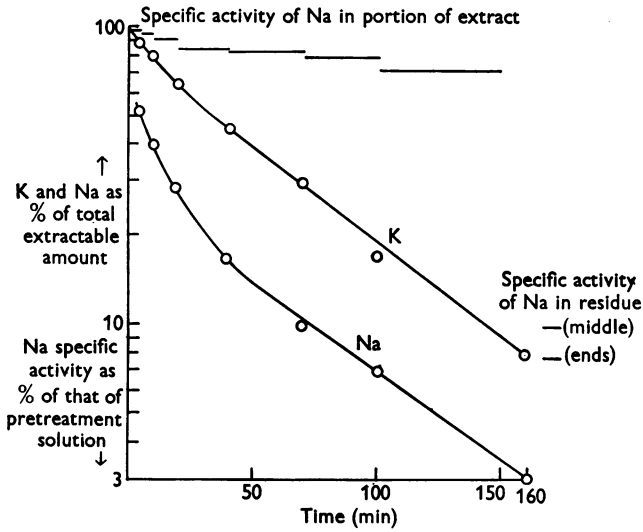

Fig. 1. Time-course of extraction of Na and K by water, with values of the specific activity of the extracted Na as percentage of the specific activity of the pretreatment solution. Total Na extracted, 39 m-equiv/kg; total K extracted, 61 m-equiv/kg. Extracellular space corresponding to fast Na fraction, 23%. Muscles loaded for 2 hr at 18° C were used.

The time-course of the extraction by water of Na and K from a pair of sartorii which had been loaded with tracer Na is shown in Fig. 1. The figure shows also the specific activities of the Na in the successive extracts relative to that of the pretreatment solution. There is a gradual diminution of specific activity during the extraction, the sodium collected between 100 and 150 min had only 72% of the starting specific activity and that in the residue was about 10%. It is to be noticed that the change in specific activity is spread over a longer time than corresponds to the escape of the fast fraction of the Na.

These values, being ratios of radioactivity to amounts of Na found by analysis, include errors from both sources. The radioactivity measurements were made for sufficient time to obtain 3% accuracy or better. The analyses could usually be duplicated to 2% by taking a quick succession of readings of unknown and standard, but the method becomes less reliable when Na concentration is low, as encountered in the terminal samples. If we claim an overall accuracy for the specific activity of within 10% at the end of the experiment the reduction seen in the terminal extract in Fig. 1 (a value of 72% of the original) is still highly significant. The lesser reduction seen during the experiment retains significance because the accuracy of the analyses improves.

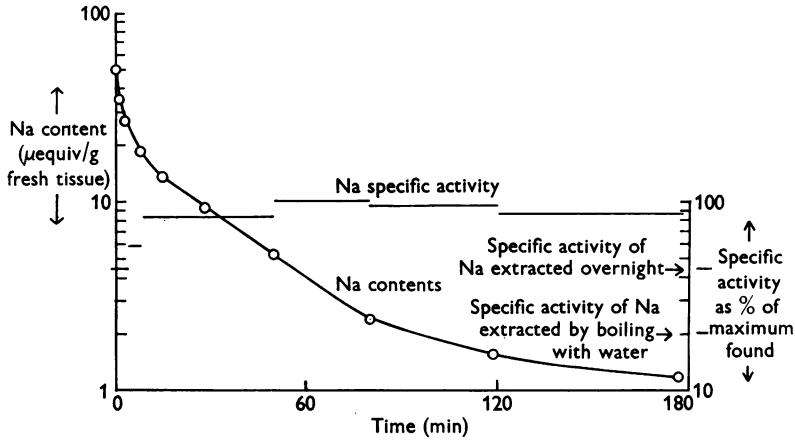

Fig. 2. Time-course of extraction of Na by water, with relative specific activities of the Na in the fractions. The muscles had been pretreated for 5 hr at 18° in tracer, then washed in ordinary saline for ½ hr at 18° C and finally extracted with water. The maximum specific activity is about 25% of that of the pretreatment solution. The fast-moving fraction of the Na (half time 1 min) corresponds to an extracellular space of 25·9%.

In order to see whether a preliminary wash in unlabelled saline would alter the pattern of the specific activity of the extracted sodium a pair of muscles were loaded for 5 hr, then washed for ½ hr in saline and finally extracted with portions of water (Fig. 2). The time-course of the loss of total Na shows a fast fraction (half time 1 min) corresponding in amount to the Na in 25·9% extracellular space. There is initially a significant rise of the specific activity corresponding to removal of the inactive extracellular Na. The specific activity reaches a maximum at 1 hr and then falls. While the reduction of specific activity of the terminal extracts is of little significance, the sodium extracted by soaking overnight, and the further quantity obtained by boiling the tissue, had specific activities significantly lower than the maximum found in the extraction.

Potassium

The degree of equilibration of muscle K which is reached depends on time of exposure, temperature and K concentration in the solution. In confirmation of previous results (Harris, 1953) it was found that 20 hr in 2·5 or 4 mM-K

at 14° C would give an exchange of 50–60%. Treatment in 12 mM-K for the same time gave 72·5% exchange.

Muscles which had been loaded with tracer K by soaking for 20 hr were examined by the water extraction procedure. The specific activity of the K in the extracts diminished, the greatest change being in the first hour, during which less than one-third of the total K is removed. A succession of samples taken between 70 and 280 min, and the residual K in the tissue itself, did not show great differences (other results are given under 'Kinetics'). No significant difference between 'ends' and 'middle' was seen (Table 2, Expts. 3 and 4).

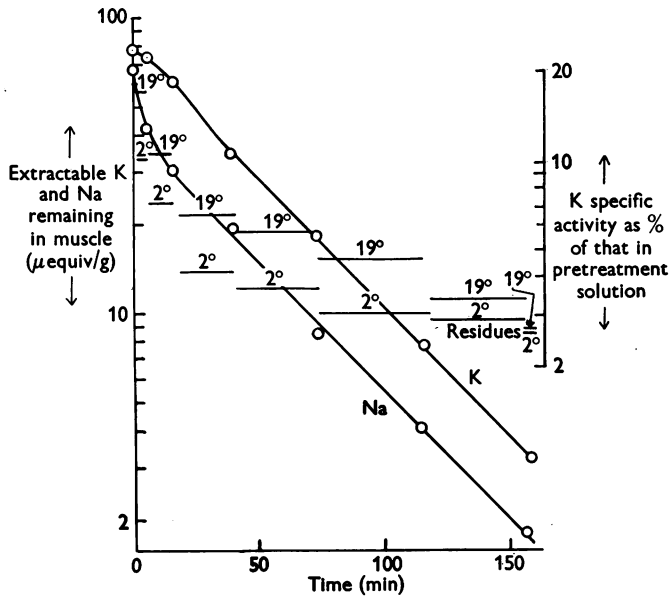

Fig. 3. Time-course of extraction of K and Na from frog sartorius plus semitendinosus muscles by water. Parallel experiments on two paired sets of muscles gave similar analytical results; one set is plotted. One set of muscles had been pretreated in tracer K (1·5 mM) for 3 hr at 19° C, the other set at 2° C. The horizontal bars show the ratios of the specific activities of the K in the water extracts to the specific activity of the K in the pretreatment solution.

It is noteworthy that there can be a large difference between the specific activity of the pretreatment solution and that of the K in the first extract. The difference between the specific activity of the fraction and that of the pretreatment solution is the greater the lower the external K concentration used, but the succession of activities is similar. Fig. 3 shows the quantities and specific activities of the K removed from sets of paired muscles which had been loaded at different temperatures (2° and 19° C) in 1·5 mM-K solution for 3 hr. As is to be expected, the total amounts of K removed are nearly identical

but the specific activities differ. It is striking that the difference of the specific activities is in the earlier portions of K extracted rather than in that remaining after 4 hr, and the K contents of the residues have undergone very similar degrees of equilibration.

Kinetics of extraction

The time-courses of the extraction of tracer and total Na and K have been observed. Fig. 4 shows the quantities of Na, K, Cl and phosphate extracted. The results are given as fractions of the amounts which water removes from the tissue in 4 hr; this is nearly all the Cl and K, but some Na and much combined phosphate remains unextracted.

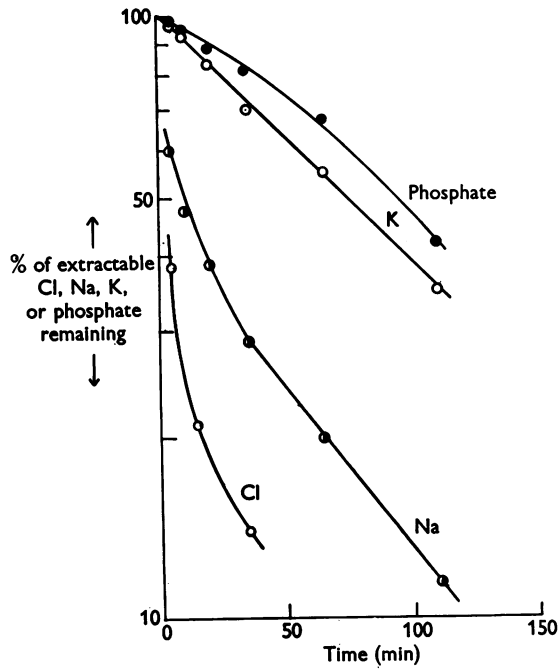

Fig. 4. Time-course of extraction of K, Na, Cl and phosphate by water. The values plotted are as percentages of the *extractable* quantities which were: K = 61·5 μequiv, Na = 35 μequiv, phosphate 23 μmole (as phosphorus), Cl = 25·2 μequiv, from 1 g tissue.

Considering Fig. 4, together with Figs. 1 and 3, the following points may be made.

(*a*) Loss of muscle K to water can be described as a first-order reaction with rate constant 0·6–1·2 hr^{-1}. The variation of rate may well be due to the different sizes of the muscles used. A lag of 10–15 min may be present before loss sets in at the full rate (Figs. 3, 4). 10–15 min is required for diffusion to clear the extracellular spaces.

(*b*) The rate constant for loss of phosphate to water is about the same as that for K loss (Fig. 4).

(*c*) After an initial rapid phase the removable part of the Na is lost at about the same rate as the K.

(*d*) The specific activity of the K falls most in the first 30 min (Fig. 3). Therefore in the first hour the loss of radioactivity is more than proportional to the loss of total K, i.e. the more exchanged K is the part which is first leached out.

(*e*) The specific activity of the Na is more nearly uniform than that of the K, but a residual fraction of Na associated with the tissue (5% of the total) undergoes little turn-over with the tracer.

(*f*) Most (85%) of the Cl is extracted in the first 30 min (Fig. 4).

TABLE 3. Cations and anions extracted from muscle by water and isotonic sugar solutions, Na, K, Cl and P measured as described in text. In calculating total anions (A^-), HCO_3^- was assumed present in one-third the chloride concentrations. P equivalents calculated as $1\frac{1}{2}$ times molar P concentrations. Initial weights of mixed muscles, 1 g each for H_2O and sugar solution. Each wash: 50 ml. for times indicated

Wash number (time, min)	Amounts extracted (m-equiv/kg fibre water)					
	Na	Na + K (B^+)	Cl	P	Cl + P + HCO_3 (A^-)	$B^+ - A^-$
			(1) Water extraction			
1 (0–5)	18·9	20·3	15·7	2·2	23·1	−2·8
2 (5–15)	6·7	12·4	4·2	6·0	11·6	+0·8
3 (15–35)	4·3	15·1	1·7	10·6	12·9	+2·2
4 (35–65)	4·0	20·7	2·0	10·9	13·6	+7·1
5 (65–110)	2·2	15·3	0·7	10·9	11·8	+4·5
6 (110–170)	1·1	12·0	0·7	5·2	6·1	+5·9
7 (170–230)	0·8	5·9	0·2	2·4	2·7	+3·2
			(2) Sugar extraction			
1 (0–5)	17·6	18·7	14·2	2·6	21·5	−2·8
2 (5–15)	6·2	7·1	4·2	0·7	6·3	+0·8
3 (15–35)	4·0	5·4	2·2	0·7	3·6	+1·8
4 (35–65)	4·2	6·7	2·0	1·5	4·2	+2·5
5 (65–110)	1·9	5·5	(5·2)	1·8	—	—
6 (110–170)	1·4	6·8	0	2·9	2·9	+3·9
7 (170–230)	0·5	5·4	0	3·4	3·4	+2·0

The first two points suggest that the loss of K is determined by the rate at which organic phosphates can break down to provide anions to accompany the K ions. Pretreatment of the muscles followed by extraction in solutions of various enzyme poisons (dinitrophenol, mercuric chloride) have not shown marked alteration of the rate.

It may be questioned whether the cell membrane survives the swelling the water causes. Muscles can be exposed for 15 min to a mixture of 2 mM each of NaCl, KCl and $CaCl_2$, and recover excitability in normal saline, with tensions up to 80% of the original. Longer exposure does cause irreversible damage. A simple and effective way to slow down the loss of K and of

phosphates is to maintain tonicity by means of sugar; either glucose or sucrose serves. Table 3 shows the time-courses of K, Na and phosphate loss to glucose. Evidently in this solution in which the cells do not swell both K and inorganic phosphate are but slowly liberated. For comparison: in water, from 1 g muscle in 1 hr K loss is 34·6 μmole, phosphate loss is 29 μmole; in sugar K loss is 5·9 μmole, phosphate loss is 5·5 μmole. In sugar the changes of specific activity of the K released from loaded muscles are but slight because so little is lost.

In water there is an initial loss of about half the total Na in 10 min, corresponding to washing out of the extracellular $NaCl + NaHCO_3$. Thereafter, the rate of loss becomes parallel with that of both K and phosphate; so it is likely to be determined by the same factor as K loss, namely the requirement of electro-neutrality.

Balance sheets have been drawn up (Table 3) for the loss of ions to successive extracts of muscle made in water and isotonic glucose. In each extraction medium there is an initial deficit of cations which, however, might well be accounted for by such ions as Mg, Ca and NH_4 which were not measured or estimated. In subsequent portions of extract there appears an anion deficit. This also may be partly accounted for by failure to measure bicarbonate (which, in our calculations was assumed to wash out in proportion to the Cl of the extracellular spaces), but it probably also indicates a loss of some organic anions comparable, perhaps, to those observed in yeast (Rothstein & Enns, 1946). The balance sheets for the two extraction media are qualitatively strikingly similar and may be interpreted as indicating that similar limiting factors (i.e. availability of diffusible anions) are determining cation leakage from muscle in both cases.

The time-courses of total sodium loss to water and to sugar are qualitatively and quantitatively similar, and also resemble the exchange of isotopic Na in saline (Fig. 5). The curves show an initial more rapid loss similar to that found for tracer Na (e.g. Harris & Burn, 1949). By extrapolation of the subsequent slower-moving fraction back to zero time it is possible to estimate the quantities of extracellular and intracellular Na, and to construct a diffusion curve for the former. Extracellular spaces so estimated were 20–26%, which correspond to values found concurrently using inulin. The slight decrease in rate of loss to water as compared to sugar and salt solutions can be partly accounted for by the increase in size of the muscle during swelling. At the time of maximum swelling (*c.* 15–30 min) the weight of the muscles may be nearly doubled with a 40% increase in mean diffusion distance.

In a pair of experiments the K concentration in the saline, or sucrose, was changed from zero to 4 mM during the run. The tracer Na output to saline was thereby accelerated, but the output to sucrose was not affected. That K ions in low concentration (4 mM) in glucose do not affect the tracer Na

movement was also shown in a pair of experiments made respectively in glucose and in glucose + KCl. The conclusion is that the part of the Na efflux which does not require K ions will go on in the absence of external ions. This means that a change from saline containing K to sugar will cause a deceleration of output since the K-contingent part of the Na efflux is stopped. The reduced rate in sugar was described by Harris (1950).

The part of the efflux which continues in sugar solution cannot be an ion exchange. The cell is the only source of ions. If the internal K ions first displaced Na ions from the peripheral sites before they themselves were shed from the cell the observation that the sum (Na + K) lost per unit time is constant (in sugar) could be accounted for.

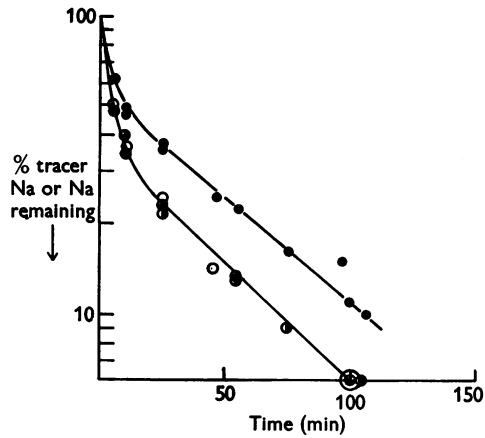

Fig. 5. Time-course of extraction of sodium (found by analysis) from muscle in water and sugar solutions, and of the exchange of tracer Na in saline solution. Two parallel experiments were made in water and are in close agreement. ●, water; ◑, saline; ○, glucose; ◒, sucrose.

Distribution of the tracer in the muscle

Diffusion in the extracellular spaces delays the exchange of the cellular K, cells in the interior of the muscle are less exchanged than those of the periphery so long as movement continues (Harris & Burn, 1949). Our results showing the non-uniform exchange of the K might then be no more than a confirmation of delay occasioned by diffusion. There are difficulties in explaining why K uptake will cease before full exchange is reached which are not accountable by diffusion alone. An attempt was made to choose between the possibilities of (*a*) non-uniform exchange across the mass of the muscle, and (*b*) non-uniformity within or between cells, by the following variations of the leaching method.

An experiment was made on a set of small muscles (average mass 20 mg).

These were loaded for 5 hr at 20° in 2·3 mM-K saline. The amounts and specific activities of the K obtained were:

Time interval (min)	Percentage of total K	Specific activity relative to pretreatment solution (%)
0– 11	15·6	56·5
11– 22	30	39
22–135	Remainder	34 (in each of three extracts)

The figures point to the exchange in all but the most easily removed K being more uniform in the small muscles.

The other variant tested was to load sartorii and then to cut longitudinal sections of the frozen muscle. The muscles were rinsed for a few minutes in K-free solution, laid flat on moist filter-paper on the freezing microtome specimen holder and frozen. One might expect to find that the first and last sections would show a higher specific activity than intermediate sections. In three such experiments where three or four major sections were obtained the K in the intermediate section(s) had a specific activity the same, or close to that of the K from the outer sections. The figures obtained were:

	Specific activities relative to that of loading solution (%)		
Conditions of loading	Outer section	Middle section(s)	Outer section
5 hr in 2 mM-K	21·4	22·6	23·3
2 hr in 4 mM-K	23·0	21·7, 22·8	24·3
6 hr in 4 mM-K	54	55	56

These values do not indicate a gross variation across the thickness of the sartorius, but they do not exclude a small quantity of high specific activity being present on the outer surfaces.

The conclusion to be drawn from these results is that non-uniform exchange is less marked in small muscles than in sartorii, but that there is no evidence for a gross variation of exchange in the thickness of the sartorius.

Nerve

Similar extraction experiments were made on medullated nerve as this material is now often the subject of tracer studies. Fig. 6 shows the time-course of the extraction of the K and Na of a desheathed frog sciatic nerve. The nerve had first been soaked for 3 hr in 2 mM-K saline containing tracer K. The specific activities of the K in the extracts were measured, and are shown on the figure. One sees that the specific activity of the nerve K is not uniform, it falls roughly in proportion to the amount of K remaining at any time: that is, the exchangeability of the K is proportional to the extractability.

This result shows that studies of the movement of tracer K from (or into) this nerve do not reveal the actual movements of K, for the internal K is not

a 'well-mixed pool'. Movement will depend upon the rate of internal mixing as well as upon membrane properties. The discrepancy between tracer and true K movements is illustrated in Fig. 7. A nerve loaded for 3 hr with tracer K was soaked in portions of K-free saline. Counts and analyses of the portions gave the data for the time-courses of tracer K and K losses. Tracer K loss shows a rapid initial phase much less evident in the K loss. A similar result, showing only 50% exchange of the later fractions of K, was found when the nerve had been exposed to the tracer solution for 16 hr at 10° C.

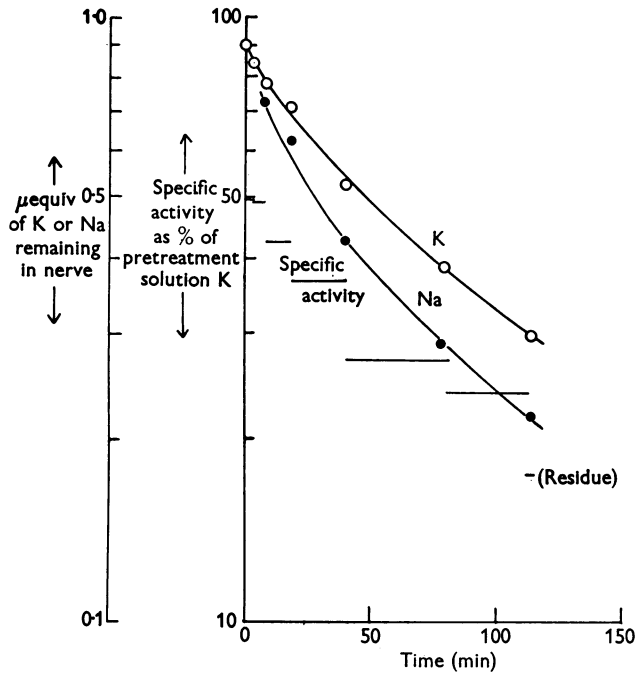

Fig. 6. The time-course of the extraction by water of K and Na from desheathed frog sciatic nerve (24 mg). The nerve had been soaked for 3 hr in 2 mM tracer K solution at 19°. The horizontal bars show the specific activities of the K in the extracts as percentage of the specific activity of the K in the pretreatment solution. Accuracy: the quantities of K appearing in successive samples are about 0·1 μequiv; analysis of these amounts is estimated to be correct to 0·01 μequiv, so the positions of the specific activity bars have an uncertainty of one-tenth of their value shown on the figure.

Swelling of the nerve is slow compared with that of muscle. Only after 8 min was the volume change obvious, and it developed in the next 20 min. The extent of equilibration of the nerve K with tracer exceeds that attained by muscle under comparable conditions.

An experiment made on desheathed nerve soaked for 2 hr in tracer Na showed that exchange of the water extractable Na was nearly complete and uniform.

Removal of Na and K by water from bull-frog nerve has been used by Shanes & Berman (1955*a*) as an analytical procedure. Their data show a behaviour similar to that which we find for these ions. From intact nerves 90% of the Na and 40% of the K was removed in 2 hr. There was 1–2 μequiv/g of both ions left in the tissue after 24 hr.

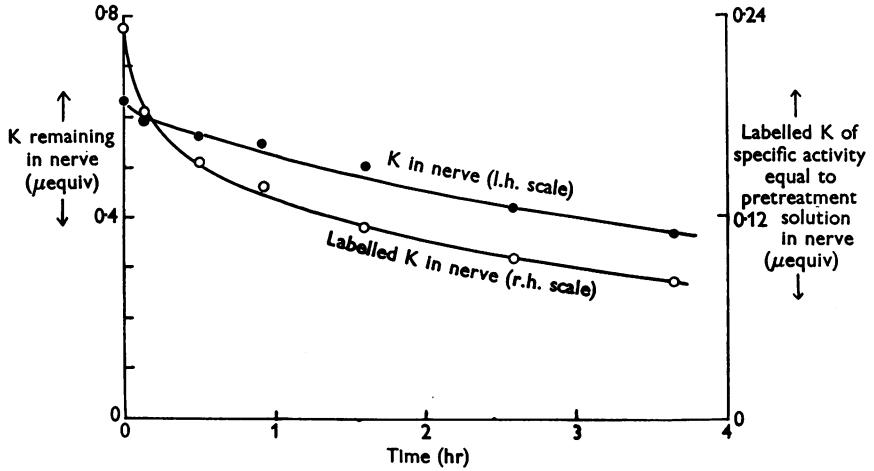

Fig. 7. The time-course of the loss from a desheathed nerve (24 mg) of tracer K and of total K to K-free saline solution. Note that different units are used for the two curves. In equivalent units the tracer K curve would lie well below the other curve as exchange was far from complete.

DISCUSSION

We shall first mention briefly the behaviour of Na, which presents the simpler case. There is a fraction of comparatively inexchangeable Na, probably associated with muscle connective tissue. How the ion is held is unknown; it may even be built in during growth. Whether the less freely exchanged Na indicated in Figs. 1 and 2 forms part of this depot is uncertain, but the existence of such material means that the results of tracer Na experiments will depend upon the time of loading (cf. Harris, 1950).

Of the intracellular Na, much is lost in the first hour of water extraction (Table 3). This behaviour would be consistent with the idea advocated by Carey & Conway (1954) that part of the muscle Na exists in a region not normally occupied by K ions with the proviso that ions in this region are either less adsorbed or more accessible than the others.

The rates of loss of Na to water and to sugar are similar, and close to the rate of exchange in saline (Fig. 5). This is remarkable since electroneutrality requires anion liberation in the first two cases and not in the third. A great difference in rate of loss of K to water and to sugar is found; comparative figures for total K extracted in 10, 40 and 70 min are: in sugar 2·3, 3·8, 5·5 μequiv/g, in water 4·6, 19, 29·5 μequiv/g. The rate of K loss is dependent

upon the generation of anions, which is faster in water than in sugar solution. In some way swelling leads to formation of phosphate groups from the muscles' store of phosphate esters.

It is striking that the ratio of Na/K in water extracts becomes nearly constant after the faster moving portion of the Na has been washed out. This can be inferred from the parallelism between Na and K curves in Figs. 1 and 3. It is likely that the ratio found between the two ions represents the true proportion holding *within* the cells. It would be of interest to examine the correlation between it and the active membrane potential. Examples of values of the Na/K ratio may be mentioned. Extracts from a muscle pre-treated in 1·5 mM-K saline had Na/K = 0·36. Exposure to media with raised K concentration (44 mM in saline or 115 mM in phosphate mixture) for 10 min led to ratios of 0·08–0·11. No evidence could be obtained for a gross change of the ratio such as might have been expected if the short pre-treatment had removed Na from an outer zone without having had time to remove it from the interior.

Treatment of muscles with water leads to 50–100% increase of volume within 30 min. During this time the muscles reversibly lose irritability and ability to contract. More prolonged treatment leads to loss of intracellular material and loss of excitability. Tobias (1950) notes, however, the persistence of a considerable membrane potential for many hours. During the initial period of water extraction Na and Cl are lost from the extracellular space. Following this K and phosphate appear in increasing amounts.

We now turn to the exchange of muscle K with tracer K. The non-uniformity of labelling we have described may be a general phenomenon. Solomon & Gold (1955) have shown that the K of erythrocytes does not behave homogeneously. Cells after exposure to tracer solution will liberate K of higher than average specific activity to a wash solution. This was interpreted as meaning that two fractions of unequal exchangeability were present.

Some variation in specific activity in any composite system will be imposed by the variable dimensions of the cells: a cell with small size and high surface/volume ratio will equilibrate faster than a larger cell. For blood cells this source of variability will be of small importance but it may be significant in muscle and nerve. Carey & Conway (1954) have discussed the distribution of fibre sizes in the sartorius in relation to the kinetics of K exchange; they, however, were concerned with a small readily exchangeable fraction (cf. Harris, 1953) which comprises 1–3% of the total K. Another source of variability of exchange is the possibility of cells differing in 'permeability'. Our results for muscle could be fitted either (i) by assuming some cells are fully exchanged and that these break down in water with a different time-course to the breakdown of other less completely exchanged cells; or (ii) that the K first extracted from all cells is the most exchanged part, leaving a residue with lower specific

activity. In (i) the amount of fully exchanged K would not have to exceed 7% of the total. The pattern of exchange against distance may either be periodically repeated from fibre to fibre, if internal mixing within the cells is slow compared with movement between exterior of the muscle mass and the interior of the mass; or the non-uniformity may reside over the whole volume of the muscle without regard to the cells. In the latter case penetration into the muscle mass must have been made slow by the outer cells acting as sinks for the tracer during its diffusion. Non-uniformity on this account should diminish with time of exposure to the tracer solution. But exchange was in fact found to be still non-uniform after 20 hr exposure.

We should like to suggest that the persistence of non-uniform exchange, and also the incomplete exchangeability of muscle K when low external K concentrations are applied (Harris, 1953; McLennan, 1955) arises because much of the internal K movement in the muscle takes place between adsorption sites in the cellular material rather than by random diffusion. Movement between sites present in the cells requires a place to be made for each ion before it can move on. Places are made in abundance when a net stream is moving in the direction of tracer movement, while tracer entry against a stream is made improbable because the ions are constantly being swept out. The suggestion will apply equally to K movement into a single cell, or into an assembly of cells between which diffusive movements are relatively slow compared with permeation. The observation that full exchange of muscle K will take place in media having raised K concentration (Harris, 1952) supports the suggestion since in high K media there is a net inward movement of K instead of more or less net loss (leak). In addition, mobile anions enter the muscle cell when it is placed in raised K concentration; these could accompany K ions in the cells permitting free diffusion to go on in parallel with site-to-site movement.

The non-uniform exchange seen in medullated nerve may be explicable in a similar way if we imagine that the major exchange is through the nodes. The tracer would then require to move along the internode. Diffusion as in free solution would lead to near equilibration of an internode 1 mm half length in 15 min, yet we find incomplete and non-uniform exchange after many hours. While we have not been able to exclude that packing of the nerve fibres does not hinder radial movement of the K into the bundle, such hindrance would be expected to show in studies of Na movement where turn-over proves to be rapid. (The half time of the slower component of Na is about 10 min at 25°, Shanes & Berman, 1955*b*.)

SUMMARY

1. The time-courses of the extraction of K, Na, Cl and phosphate by water and by sugar solutions from frog muscle have been examined.

2. Similar experiments have been made using muscles which had first been loaded with either radioactive Na or K. The cation contents and radioactivities of the extracts were measured.

3. It is shown that most of the Na extracted from muscles loaded for over 2 hr is of nearly uniform specific activity (radioactivity/unit quantity Na) but some Na remains in the muscle residue which is only slightly radioactive. This part of the Na is found more at the ends of the muscle than at the middle, suggesting that it is present in connective tissue.

4. The radioactive K extracted from muscles which have been loaded either for 3 hr or 16 hr is not of uniform specific activity, the first extracts (*c.* 30 min in water) contain K of higher specific activity than subsequent extracts, but all extracts show a lower specific activity than that of the loading solution. The degree of exchange is increased as the external K concentration is increased.

5. The K and the intracellular part of the Na are accompanied by phosphate ion in the extracts. Some additional anion is also required to preserve an electrical balance. The rate of extraction of the cations may be determined by the rate of liberation of phosphate from organic compounds.

6. Experiments on desheathed sciatic nerve show that the K in this tissue also does not attain a uniform specific radioactivity.

7. A consequence of the non-uniformity of exchange of muscle and nerve K is that the movement of tracer K will not be a measure of the trans-membrane flux, the rate of internal mixing also being involved. It is suggested that the K ions may be held on a matrix of adsorption sites in the cells; this can explain the observed failure of the tracer K to mix fully and to undergo complete equilibration with the K in the tissue.

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