THE TRANSFER OF POTASSIUM BETWEEN MAMMALIAN MUSCLE AND THE SURROUNDING MEDIUM

by

H. McLENNAN

Donner Laboratory of Experimental Neurochemistry, Department of Neurology and Neurosurgery, McGill University, and the Montreal Neurological Institute, Montreal (Canada)

There are in the literature a number of reports on the movement of sodium and potassium ions across the boundaries of muscle cells; these investigations have however largely been confined to frog muscle. CREESE[1] and GOURLEY AND JONAS[2] have investigated the K exchange in mammalian (rat) muscle *in vitro:* these authors have reported that all of the muscle K is capable of undergoing exchange with the K of the medium in which the muscle is incubated. HEVESY AND HAHN[3] and NOONAN, FENN, AND HAEGE[4] have studied the uptake of K by muscle *in vivo* following injection of ^{42}K. The latter group found that the whole of the muscle K was exchanged under these conditions, while the former found that only a portion of the K will so exchange. HAHN AND HEVESY[5] further showed that the proportion of K undergoing exchange could be increased by muscular excercise; complete exchange was however still not attained.

As a preliminary to the investigation of the effects of certain drugs and other conditions on the normal K movements, it appeared necessary to study more fully the transport of K in normal mammalian muscle *in vitro*. This has been done with muscles of two different shapes, incubated in Ringer-type solutions and in solutions of K phosphate; under the latter condition the K content of the muscle cells tends slowly to increase, in contrast to the former where the tendency is to a decrease. The use of phosphate solutions eliminates swelling of the tissue during incubation, since the cell wall is only slowly permeable to phosphate ion.

EXPERIMENTAL

Materials: The muscles used in this study were the extensor digitorum longus and the sartorius of young hooded rats. The former muscle is easily dissected without damage to the tissue; cotton loops were tied around the tendons at each end of the muscle prior to its removal from the body. The sartorius of rodents is closely fused to the tensor fasciae latae (GREENE[6]). In young animals it was, however, found possible to dissect the sartorius with minimal damage to its margins; again loops were attached to the ends of the muscle before it was removed. After excision the muscles were weighed on a torsion balance. At the end of the experiment the loops were cut off and weighed separately, and the final weight of the muscle was also determined.

Radioactive ^{42}K was obtained in the form of carbonate from the Brookhaven National Laboratory, Upton., U.S.A.; and from Atomic Energy of Canada, Limited, Chalk River, Ont.

Methods: In order to measure the uptake of K from a saline solution, the muscle with its attached loops was placed in a large volume, *ca.* 50 ml, of solution of the following composition: for 20° C, HCO_3 37, Cl 103.5; for 37° C, HCO_3 24.5, Cl 116; plus in both cases Na 131.5, K 5, Ca 2, Mg 2, SO_4 2, glucose 10 (all in mmol/l). The pH of these solutions when bubbled with 95% O_2–5% CO_2 was near 7.4. Any changes in the K content of these media, such as were necessary for some experiments, were

References p. 95.

balanced by changes in the Na concentration in order to preserve isotonicity. To prepare a solution containing ^{42}K, the $^{42}K_2CO_3$ was neutralized with HCl, made up to give an isotonic solution (154 mmol/l), and used in place of the inactive KCl solution in the preparation of the above media.

K phosphate solution was prepared by adding to the $^{42}K_2CO_3$ enough dilute H_3PO_4 to give a solution which contained 225 mmol/l K and 150 mmol/l phosphate. On the day of receipt of the isotope this phosphate solution was diluted 20 × with an inactive solution of the same composition; the dilution with inactive material was reduced as decay diminished the radioactivity. The phosphate solution had a pH of about 7.0.

The radioactivity of the muscle was measured at various times during incubation by removing the tissue from the solution and washing it for exactly 1 min in inactive medium of the same composition in order to remove adhering drops of radioactive saline. The cotton loops were placed over pegs set in a flat plastic base, and the whole slid beneath an end-window Geiger tube (Tracerlab type TGC–1). This method ensured a reasonable degree of reproducibility with respect to the geometrical position of the muscle under the counter. Counts of the muscle radioactivity were determined for three 1 min periods, at the end of which time the muscle was returned to the radioactive medium.

After a sufficient period of soaking in radioactive medium (at least 4 h), the rate at which the radioactive K was lost from the muscle could be followed by transferring the tissue to an inactive solution of the same composition, and assaying the radioactivity at intervals as above. The only change required in the procedure was that it was no longer necessary to wash the tissue prior to assay, since the fluid in which the muscle was now placed had a negligible activity.

At the end of the experiment the muscle was dissolved in a few drops of HNO_3 freshly distilled in silica vessels. An aliquot of the solution so obtained was assayed for radioactivity, and this activity compared with that of the radioactive saline used in the first half of the experiment, thus providing a direct relation between number of counts and amount of K.

All radioactivity measurements were corrected for blank, decay, and resolution time of the scaler-Geiger counter combination.

K analyses were performed with the aid of a flame photometer (Perkin-Elmer type 52C). The nitric acid solution of the muscle was taken to dryness, the residue taken up in water and an aliquot appropriately diluted for the measurement.

RESULTS

In the investigation of the rate of movement of an ion it is of importance first of all to determine whether the time barrier imposed between equilibration of the ions inside and outside the tissue is due to the resistance of the cell boundaries or to some diffusion barrier. In the case where the rate of transfer of an ion is determined by the permeability of the cell boundary, the probability of entry of an ion will be proportional to the external concentration of that ion $[x_o]$; the probability of transfer from inside to outside will be proportional to the internal concentration $[x_i]$. In a cell with surface area A, then, the net amount of ion entering will be $\varkappa_1 A[x_o] - \varkappa_2 A[x_i]$ per unit time, $\varkappa_1$ and $\varkappa_2$ being constants. If the volume of the cell is V, the rate of increase of concentration inside will be $(\varkappa_1[x_o] - \varkappa_2[x_i])A/V$. Putting $\varkappa_1 A/V = k_1$ and $\varkappa_2 A/V = k_2$, we have

$$\frac{d[x_i]}{dt} = k_1[x_o] - k_2[x_i],$$

which is the normal permeability equation. It will be noted that the transfer rate constants k_1 and k_2 are independent of the number of cells in the aggregate comprising the tissue, and vary only with the surface/volume ratio of the cells. If, therefore, the rate constants determined experimentally are found to vary with the size or shape of the tissue sample employed, then the limiting factor may be expected to be the rate of diffusion of the ions into the interstices of the tissue.

In the present experiments an attempt has been made to decide between these two possibilities by using two muscles of different shape, although of about the same overall volume. The extensor digitorum longus is nearly circular in cross-section, tapering somewhat towards each end; the sartorius is a thin flat sheet of muscle of fairly uniform

References p. 95.

width. Both have an average total volume of about 100 mm³, and both have fibres of 30 μ diameter for the most part. Assuming that the fibres are cylindrical and run the whole length of the muscle (30 mm for the extensor), the volume of an "average" fibre will be 0.021 mm³, and its surface area 2.83 mm².

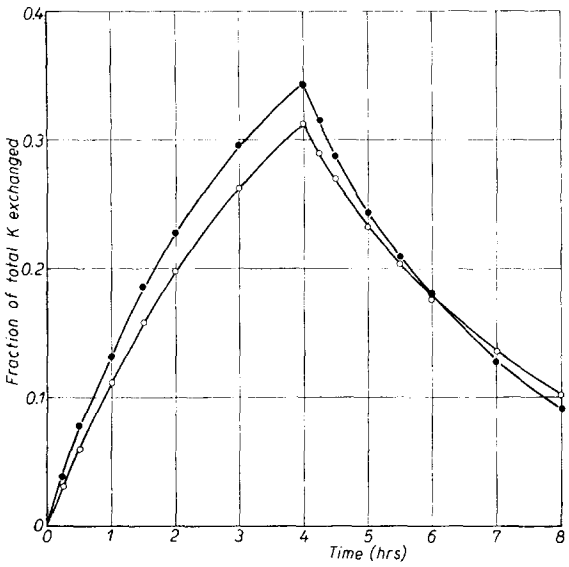

Fig. 1. The exchange of ^{42}K in two muscles from the same rat. Temperature 20° C. External K concentration 5 mmol/l. After 4 h the muscles were permanently transferred to inactive medium.

—○——○— Extensor digitorum longus.
—●——●— Sartorius.

Fig. 1. shows the results of typical experiments in which the uptake of K from a Ringer-type solution containing ^{42}K was measured, and where after some hours the muscles were placed in an inactive solution of the same composition and the time course of the loss of radioactivity followed. The figure also gives a comparison between the extensor digitorum longus and the sartorius. Empirically the uptake curves can be fitted by a simple exponential function of the type $K^* = B(1-\exp(-bt))$, where K^* is the amount of labelled K in the tissue, B is a constant representing the fraction of the total tissue K which is undergoing exchange, and b is the time constant governing the process. Strictly the equation for K^* should involve two terms, *i.e.* one for the extracellular K as well as the above, but as will be discussed below the amount of K contained in the extracellular space in these muscles is too small to be detected within the overall errors of measurement. If a steady state with respect to the total K content of the tissue pertains, then according to the superposition theorem the equation for the loss of K^* when the tissue is placed in an inactive medium at time t_1 should be

$$K^* = B(1 - \exp(-bt)) - B(1 - \exp(-b(t - t_1))).$$

With the medium used in these experiments the K content of the tissue remains reasonably constant. The average K content of fresh muscles was 101 ± 13 μequiv/g (S.D., 6 determinations); that of muscles incubated for periods up to 8 hr 96 ± 10 μequiv/g (S.D., 9 determinations): it has further been found that the average rate of net loss of K was less than 2% of the total per h at 20° C for periods of incubation up to 16 h. It is therefore permissible to use the steady state equations. In the experiments shown in Table I the constants B and b were derived from equations of the above type, which fitted the experimental curves closely.

From Table I it is clear that the time constant b governing the movement of about one half of the total tissue K is of the same order of magnitude for both of the muscles investigated. It may be inferred, therefore, that the constant describes a permeability rather than a diffusion process. If diffusion were the limiting factor determining the penetration of K, then the rate of penetration into the sartorius would be expected to

References p. 95.

be greater than into the extensor digitorum longus, inasmuch as the thickness of the sartorius is of the order of 0.5 mm, while that of the extensor is 3.5 mm.

TABLE I

PARAMETERS OF THE EQUATIONS FOR UPTAKE OF LABELLED K FROM A RADIOACTIVE SALINE SOLUTION BY RAT MUSCLES, AND FOR THE LOSS OF LABELLED K FROM MUSCLES TO AN INACTIVE SOLUTION. For equations, see text. Temperature 20° C. External K concentration 5 mmol/l

B as fraction of total tissue K; b in h^{-1}.

Extensor digitorum longus		*Sartorius*	
B	b	B	b
0.49	0.22	0.37	0.41
0.59	0.26	0.47	0.33
0.41	0.40	0.37	0.19
0.42	0.41		
0.46	0.17		

The effect of changing the K concentration of the bathing solution upon the time constant b and upon the amount B of tissue K subject to this time constant is shown in Table II. Attempts were made to perform experiments of this type also at 37° C. At this temperature, however, the total K content of the tissue tended slowly to fall to about one half of the initial value (45 μequiv/g instead of 96 μequiv/g) after 8 h incubation. It is therefore not permissible to apply the steady state equations given above. It was found that qualitatively the picture is the same at 20° as at 37°, but the value of b falls from an average of 0.52 h^{-1} at 37° to 0.32 h^{-1} at 20° (both with 5 mmol/l external K).

The results in Table II show that the effect of increasing the external K concentration is to raise the amount of K which undergoes exchange, without at the same time increasing the rate at which the process is carried on. As the K concentration in the medium is raised, the exchangeable fraction of the K is raised until in a concentration of 20 mmol/l the exchange is almost complete.

TABLE II

FEFECT OF EXTERNAL K CONCENTRATION ON THE TURNOVER OF RAT MUSCLE K AT 20° C. PARAMETERS B AND b DETERMINED BOTH FROM MEASUREMENTS OF INFLUX AND EFFLUX

B as fraction of total tissue K; b in h^{-1}

Extensor digitorum longus			*Sartorius*		
K conc. mmol/l	B	b	*K conc. mmol/l*	B	b
5	0.45	0.17	5	0.37	0.19
10	0.61	0.22	10	0.72	0.20
20	0.89	0.27	20	1.00	0.36
5	0.46	0.28			
10	0.59	0.26			
20	0.88	0.24			
10	0.66	0.17			
20	0.77	0.24			

References p. 95.

The figure which it is of the most interest to know is the amount of ion being transferred per unit of cell surface. An estimate of this may be obtained by considering the dimensions of an "average" fibre of the extensor muscle given above, *i.e.* a volume of 0.021 mm^3 and a surface area of 2.83 mm^2. With the muscle in a steady state the influx of K per unit time must equal the efflux, that is

$$k_1[K_o] = k_2[K_i].$$

Now the first order equation describing the disappearance of a reactant from a system is

$$-\frac{dc}{dt} = k_2 c,$$

where c is the concentration of the reacting substance. This can be integrated in the form $c_t = c_o \exp(-k_2 t)$, c_o and c_t referring to the concentrations of c initially and at time t. This equation can be applied to the present problem in the form $K^*_t = B \exp(-k_2 t)$, whence it appears that the values of b determined above are identical with $k_2 \cdot [K_i]$, the internal K concentration, may be found from B, and $[K_o]$ is also known. For 5 mmol/l external K concentration, then,

$$k_1 = \frac{k_2\,[K_i]}{[K_o]} = \frac{0.32 \cdot 38}{5} = 2.43\ h^{-1} \text{ (average of 4 measurements).}$$

The amount of K being transferred per unit of cell surface is equal to the flux of K $\times$ the ratio V/A. That is,

$$\text{Influx/mm}^2 = k_1\,[K_o]\,V/A = \frac{2.43 \cdot 5 \cdot 10^{-3} \cdot 0.021}{2.83 \cdot 3600} = 2.5 \cdot 10^{-8}\ \mu\text{equiv/mm}^2\text{/sec}$$

With an external K concentration of 10 mmol/l (5 determinations), the corresponding values are $k_1 = 1.18\ h^{-1}$, flux $= 2.4 \cdot 10^{-8}$ μequiv/mm^2/sec; and with 20 mmol/l external K (3 determinations), $k_1 = 0.94\ h^{-1}$, flux $= 3.9 \cdot 10^{-8}$ μequiv/mm^2/sec. Thus although the external K concentration is greatly increased, the amount of ion being transported across the cell boundary is not much raised. It may be that the system responsible for K transport is normally working at near capacity, and large changes in the external K concentration produce only small alterations in the total K flux.

The extracellular space of the rat gastrocnemius is given as 12% by volume (MANERY AND HASTINGS[7]); assuming that the concentration of ion in this space is the same as that in the external solution at equilibrium, the amount of K which would be expected in the extracellular phase of these muscle is 0.6 μequiv/g of tissue, *i.e.* less than 1% of the total K. In experiments involving the exchange of K with a Ringer-type solution, this small amount of K exchanging in the extracellular space is unlikely to be capable of measurement. Nevertheless the equation covering the overall K movement must consist of two terms; one a diffusion term for the equilibration of the extracellular fraction, and the second a permeability term for the movement of the ion across the cell wall. Only if the time constants governing these two processes are substantially different can movement in the two phases be dissociated.

Experiments with K phosphate made it possible to measure the volume of the extracellular space of the muscle as well as the rate of turnover of the extracellular K. These experiments were performed as follows. The muscle was placed in a solution of K^* phosphate for 1–2 h, at which time all the tissue K determined by analysis had undergone

References p. 95.

exchange. In addition, the normal high Na content of the extracellular space had been replaced by the K* ions of the solution. The muscle was then transferred to a solution of K* Ringer of the same specific activity, and the tissue radioactivity was determined at short intervals. The result was a sudden outpouring of the K* of the extracellular space in exchange for Na; the intracellular K on the other hand remained almost constant or fell very slowly, since the intracellular K level in K phosphate was found to be slightly above that found after incubation in saline medium. In three experiments of this type the extracellular space of the extensor muscle was found to be 0.10–0.15 of the total volume, and the K contained in this space turned over with a half time of 5–7 mins. This rate of exchange is very much faster than that of the intracellular K, where the half time is of the order of 1½ h. A typical experiment is shown in Fig. 2.

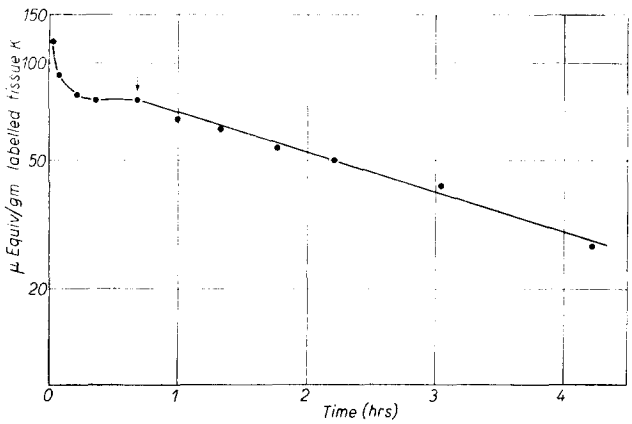

Fig. 2. Effect of incubation for 1.3 h in K* phosphate solution (external K concentration 225 mmol/l) At time o the muscle was transferred to K* Ringer (external K concentration 5 mmol/l) of the same specific activity; at the arrow the muscle was permanently transferred to inactive Ringer.

Having made the above measurements, the muscles could then be transferred from K* Ringer to inactive Ringer of the same composition, and the time constants for the efflux of intracellular K determined in the usual way. Pretreatment with K phosphate did not cause any change in the rate of K efflux in saline solution; however the K, which in saline was inexchangeable (see Table I), and which had been rendered "accessible" by the application of K phosphate, did not appear to become re-fixed when the muscle was returned to saline solution. In other words, B, the amount of exchangeable K in the intracellular phase, now approached unity. The results are given in Table III.

TABLE III

VALUES OF THE PARAMETERS B AND b DETERMINED FOR THE EFFLUX OF K FROM MUSCLE TO AN INACTIVE RINGER SOLUTION AFTER TREATMENT OF THE TISSUE WITH K* PHOSPHATE

Temperature 20° C. B as fraction of the total tissue K; b in h^{-1}.
All measurements on extensor digitorum longus.

B	b
0.93	0.26
0.92	0.24
0.91	0.38
0.93	0.32
0.95	0.23

That this effect was not due to the presence of a high concentration of phosphate was shown by the fact that pre-incubation of the muscle in a solution of Na phosphate of the

References p. 95.

same concentration produced no alteration in the subsequent uptake of K^* ($B = 0.44$, $b = 0.34$ h^{-1}). Pre-incubation for 1 h in inactive K phosphate caused an increase in the value of B to 0.71 when the influx of K^* was measured subsequently.

The effect of stretch on frog muscle metabolism was investigated by FENG[8], who found that the resting metabolism was increased by the application of tension. Fig. 3 shows the effect of applying stretch on the K transport of the extensor digitorum longus. The stretch was given to 115% of the resting length by slipping the loops at the ends of the muscle over a U-shaped wire frame, such that the separation at the mouth of the U could be varied, and with it the length of the muscle. Otherwise the experiment was performed as before. Three such experiments were done at 20° C and one at 37°, all with the same lack of effect on either K influx or efflux. This is in accord with the results of HARRIS[9], who found a similar lack of effect of stretch on K movement in frog muscle.

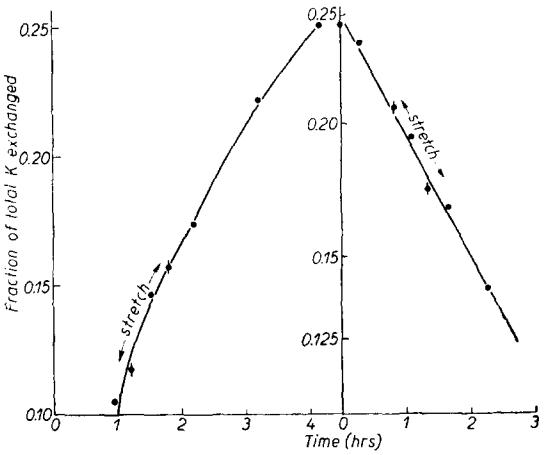

Fig. 3. The effect of stretch on K exchange in muscle. Stretch to 115% of the resting length applied between the times indicated.

DISCUSSION

These experiments, which were designed to provide information on the movement of K in resting mammalian muscle fibres *in vitro*, have shown that qualitatively there is little difference between rat muscles and those of the frog (HARRIS[9]). Thus in both species the exchange of the total tissue K is incomplete if the tissue is incubated in saline medium containing a physiological concentration of K, complete exchange only being attained if the external K concentration is much increased.

That the measured K exchange in these muscles is not being limited by a diffusion barrier is indicated both by the fact that the rate and extent of the exchange is the same in muscles of differing shape (extensor digitorum longus and sartorius), and as well from the determination of the movement of the extracellular K after incubation in K phosphate. At 20° C this equilibration of fast moving K is 50% complete in about 6 mins. Assuming that the extensor is a cylinder of radius $r_0 = 0.18$ cm, then half saturation is reached at time t when $kt/r_0^2 = 0.062$ (HILL[10]), where k is the diffusion constant governing the process. From this equation it can be calculated that $k = 5.6 \cdot 10^{-6}$ cm^2/sec. The diffusion constant for K ions in free solution is given as $1.6 \cdot 10^{-5}$ cm^2/sec at 17.5° C for 0.1 M KCl; the diffusion of K into the extracellular space of these muscles is thus only reduced to one third of that holding in free solution. The fact that the bulk of the tissue K exchanges much more slowly than this is indicative of the permeability barrier imposed between the cellular contents and the extracellular space.

Values reported in the literature for the cell permeability constant $\varkappa_1$ of rat muscle *in vivo* vary between 1.6 and $5 \cdot 10^{-2}$ cm/h (calculated from HAHN AND HEVESY[5], and

References p. 95.

NOONAN, FENN AND HAEGE[4]). The value of $\varkappa_1$ in the present experiments can be calculated from the values obtained for k_1, since $k_1 = \varkappa_1 A/V$. The figure for $\varkappa_1$ so obtained is $1.8 \cdot 10^{-3}$ cm/h at 20° C, the difference between this and the older values being partially due to the lower temperature. Since the uptake of K by the cell against the gradient of electrochemical potential is an active process, the temperature coefficient may be presumed to be high.

The finding that with an external K concentration of 5 mmol/l some 50% of the tissue K fails to undergo exchange with the ions of the solution requires some consideration. The fact that the time constants b determined both for uptake of K from a radioactive solution and for the loss of K from a labelled muscle to an inactive solution agree well with eachother means either that the constant cannot govern the movement of all of the intracellular K, or else that there is a large imbalance between influx and efflux. The second alternative could only mean that a *net* loss of K was taking place of such a magnitude that half of the total K would be lost during three hours of incubation. This has not been observed to occur. A further proof was given by an experiment in which a pair of muscles were incubated overnight, one in radioactive saline and the other in inactive. In the morning the second muscle was placed in active medium, and the time course of the uptake of K measured in the usual way; at the same time the first muscle was continued in active medium to ensure that no further K exchange was taking place. The results were: muscle incubated overnight in radioactive medium, $B = 0.46$; muscle pre-incubated in inactive medium, $B = 0.41$, $b = 0.40$ h^{-1}. The extent to which the K of the muscle was able to undergo exchange was thus not affected by a 10 h pre-incubation; the value of b is also within the limits found for "fresh" muscles. It seems certain, therefore, that there is a fair proportion of the cellular K of muscle which is not free to exchange *in vitro*. Whether raising the temperature would have the effect of increasing the proportion of exchangeable K, as is the case with frog muscle[9], cannot be determined from these experiments, where incubation at 37° brought about the gradual loss of tissue K. It is interesting in this connection that STONE AND SHAPIRO[11] have reported that one third of the total K of rat muscle is not ultrafilterable.

SUMMARY

The rates of uptake and loss of tracer-labelled potassium by isolated muscles of the rat have been studied. The rates were found to be of the same order in two muscles (extensor digitorum longus and sartorius) of different shape but of similar total volume.

In Ringer's solution at 20° C one half of the total tissue K exchanged with first-order kinetics, the remainder being apparently inexchangeable. The proportion of exchangeable K could be increased by increasing the K concentration of the bathing medium, complete exchange being attained with a concentration of 20 mmol/l.

The rate of exchange of the K contained in the extracellular space of the tissue, and the volume of the space, were measured following incubation of the tissue in isotonic K phosphate solution. The extracellular K exchanged with an average half time of 6 min, in contrast to the 1½–2 h period required for half exchange of the exchangeable intracellular K.

The application of mechanical stretch was without effect on the influx or efflux of K in the muscle.

RÉSUMÉ

Les vitesses d'entrée et de sortie du potassium dans les muscles isolés de rats ont été étudiés au moyen de potassium marqué. Les vitesses étaient semblables pour deux muscles de forme différente mais de même volume (extensor digitorum longus et sartorius).

Dans une solution de Ringer, la moitié de la quantité totale de K présente dans le tissu fut échangée suivant la cinétique d'une reaction du premier ordre, le reste étant apparemment non

References p. 95.

échangeable. La proportion du K échangeable put être augmenté par élévation de la concentration du milieu extérieur en K. Lorsque cette concentration était de 20 mmol/l la totalité du K fut échangé.

Le volume de l'espace extracellulaire et la vitesse d'échange de son K ont été mesurés après incubation du tissu dans une solution isotonique de phosphate de K. Le K extracellulaire s'échangeait avec une demi-periode moyenne de 6 minutes alors qu'elle est de 1½-2 heures pour le K intracellulaire échangeable.

Une tension mécanique exercé sur le muscle fut sans effet sur l'entrée ou la sortie du K.

ZUSAMMENFASSUNG

Die Geschwindigkeit der Aufnahme und der Abgabe von markiertem K wurde an isolierten Muskeln der Ratte studiert. In zwei Muskeln von verschiedener Gestalt aber gleichem Volumen (Extensor digitorum longus und Sartorius) ist die Geschwindigkeit des K-Austausch von der gleichen Grössenordnung. In Ringer Lösung bei 20° C wird die Hälfte des Gesamten K des Gewebes ausgetauscht in einer kinetischen Reaktion erster Ordnung, der Rest scheint nicht austauschbar. Das Verhältnis des austauschbaren K konnte erhöht werden, durch Erhöhung der K-Konzentration im Bad, vollständiger Austausch wurde erhalten bei einer Konzentration von 20 mmol/l.

Die Austauschgeschwindigkeit des K, in extrazellulären Gewebsspalten und ihr Volumen wurden nach Einlegen des Gewebes in isotonische K-Phosphat-Lösung gemessen. Der extrazelluläre K-Austausch mit einer Halbwertszeit von 6 Min. steht im Gegensatz zu der Zeit von 1½ bis 2 Stunden für den Austausch des intrazellulären K.

Die Anwendung mechanischen Zugs blieb ohne Wirkung auf die Aufnahme und die Abgabe von K im Muskel.

REFERENCES

1 R. CREESE, *J. Physiol.*, 115 (1951) 23P.
2 D. R. H. GOURLEY AND H. JONAS, *Fed. Proc.* 13 (1954) 59.
3 G. HEVESY AND L. HAHN, *Klg. Danske Videnskab. Selskab. Biol. Medd.*, 16 #1 (1941).
4 T. R. NOONAN, W. O. FENN AND L. HAEGE, *Am. J. Physiol.*, 132 (1941) 474.
5 L. HAHN AND G. HEVESY, *Acta Physiol. Scand.*, 2 (1941) 51.
6 E. C. GREENE, Anatomy of the Rat, *Trans. Am. Philosph. Soc.* (1935).
7 J. F. MANERY AND A. B. HASTINGS, *J. Biol. Chem.*, 127 (1939) 657.
8 T. P. FENG, *J. Physiol.*, 74 (1932) 441.
9 E. J. HARRIS, *J. Physiol.*, 120 (1953) 246.
10 A. V. HILL, *Proc. Roy. Soc.*, B 104 (1929) 39.
11 D. STONE AND S. SHAPIRO, *Am. J. Physiol.*, 155 (1948) 141.

Received July 12th, 1954