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Studies of Caesium Uptake by Rat Soleus and Vastus lateralis Muscles *in vivo* and of Its Efflux Rate Relative to Potassium *in vitro*

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Summary. In rats given drinking water containing 40 mMCsCl for periods of up to 15 days, uptake of Cs^+ into fibres was much more rapid in red soleus than in pale vastus lateralis muscles. On the 3rd day the concentration gradient between fibre water and plasma was 2.5 times greater for Cs^+ than for K^+ in soleus while in vastus the former had not yet reached equality with the latter. On the 5th day mean membrane potential measured by microelectrode *in vivo* was −78 mV in vastus and −79 mV in soleus. Rate coefficients for net efflux of Cs^+ and K^+ from isolated Cs-enriched muscles into anaerobic K-free Krebs containing ouabain were not significantly different from one another in several muscle types examined. When Cs-rich muscles were immersed for 30 min in plasma from the same animal containing ouabain and bubbled with nitrogen and membrane potentials were measured in addition to net efflux of Cs^+ and K^+, the calculated permeability coefficients for K^+ efflux was about 4 times that of Cs^+ in all muscles examined. The results suggest that the very striking accumulation of Cs^+ in red muscles in contrast to pale ones was not due to slower efflux of Cs^+ relative to K^+ in the former muscles, but to a much faster influx rate for this cation.

Key words: Rat Muscle — Net Fluxes — Caesium.

Relman and his colleagues (1957) made rats potassium deficient by feeding them on a diet lacking this cation for a period of a week. They then gave them drinking water containing mixtures of potassium and rubidium or caesium salts for a further week or two after which they sacrificed them and analysed unspecified muscles and blood plasma for these cations. They found that as much as two thirds of the cellular potassium had been replaced by caesium or rubidium. When they expressed the ionic gradients across the muscle fibre membrane as concentrations in muscle fibre water divided by those of the plasma, they obtained values of 40, 115 and 216 for potassium, rubidium and caesium, respectively. They concluded from these observations that the ions were probably actively transported into the fibres and that passive uptake was unlikely unless selective binding with negligible change in osmotic pressure took place.

It was subsequently found (Kernan, 1969) that it was the red tonic muscles such as the soleus which accumulated caesium and rubidium preferentially, while paler phasic muscles including the vastus lateralis had similar ionic gradients for potassium, rubidium and caesium between muscle fibre water and plasma. In our experiments the rats had not been made potassium-deficient prior to exposure to drinking water containing either rubidium or caesium chloride (20 mM) for 2 weeks. The most striking example of selective uptake of rare alkaline metal ions which we found was caesium accumulation in red soleus muscle fibres. At the end of a period of 2 weeks the ratio of caesium concentration in fibre water divided by its concentration in plasma water was about twice as great as the corresponding ratio for potassium ions. Since the potassium ions were probably passively distributed across the fibre membrane in a Donnan equilibrium and close to equilibrium with the resting membrane potential it followed that caesium ions were far removed from electrochemical equilibrium across the membrane. The apparent absence of equilibrium with respect to caesium might be explained if a large fraction of this cation was selectively bound within the cell, otherwise it would seem necessary to suggest that caesium was being actively pumped into the cell. In the pale muscles including the vastus lateralis where the distribution of potassium, rubidium and caesium ions across the fibre membrane were similar and apparently in equilibrium with the resting potential it would seem likely that caesium and rubidium entered passively towards a Donnan equilibrium.

On the basis of these results it was suggested that soleus muscles might normally accumulate potassium almost exclusively by active carrier mediated transport perhaps because of low membrane permeability to this cation. Caesium might then effectively compete with potassium for carrier sites but diffuse out of the fibres passively through permeability channels more slowly than potassium. In phasic muscles on the other hand it was suggested that potassium might normally be taken up passively through permeability channels, leaving the fibres in the same manner thus reaching equilibrium with a potential across the membrane established perhaps by an electrogenic sodium pump (Kernan, 1962; Adrian and Slayman, 1966). In such a system caesium and rubidium ions would be expected to approach a similar condition of equilibrium.

In this paper the characteristics of caesium accumulation were compared in soleus and vastus lateralis muscles as representing the red and pale muscle types, respectively. Mean resting potentials were then determined *in vivo* by means of the microelectrode in both soleus and vastus muscles of caesium enriched rats and these were compared with the calculated equilibrium potentials of the caesium and potassium ions across the fibre membrane. The reason for this was to ensure that mem-

brane potentials were comparable in both muscles and that no hyperpolarization occurred in the soleus muscles which might account for its greater concentration gradient for caesium between plasma and fibre water.

In order to examine the possibility that a slower rate of efflux of caesium relative to potassium might have contributed to the accumulation of the former cation in soleus muscle fibres, net efflux rates for the two cations from caesium-enriched muscles were compared in various muscles.

Finally relative rates of uptake of potassium and caesium by soleus muscles were measured *in vitro*, in experiments similar to those of Conway and Moore (1945) where cation uptake was determined by increase in muscle weight.

Methods

The rats used were of the Wistar strain. In examining the rates of accumulation of caesium by the muscles the rats were given drinking water containing 40 mM-CsCl for a period of up to 15 days in addition to their normal solid diet. Animals were sacrificed on the 3rd, 5th, 9th and 15th days after exposure to the caesium solution. They were first anaesthetised with ether and blood was removed for analysis as already described (Kernan, 1969). The cation concentrations in muscles and in blood plasma were measured by means of the Zeiss flame photometer which gave very good resolution between the caesium emission line at 852 mμ and that of potassium at 768 mμ. The distribution of water within the muscles was measured as already described (Kernan, 1969) and the cation concentrations were expressed as milliequivalents per litre muscle fibre water and in the case of plasma as milliequivalents per litre plasma water.

In examining the short term accumulation of caesium, rats were anaesthetised with ether and the small intestine exposed through an opening in the medial abdominal wall. About 10 ml of a 40 mM caesium acetate solution was injected slowly into the gut and the animal was sutured and allowed to recover. The rats were again anaesthetised 6 h later for removal of blood and were then sacrificed before excision of the muscles for analysis. The muscles used were the soleus and vastus lateralis.

Membrane Potential Measurement in vivo. In the measurement of the mean resting potentials of the soleus and vastus muscles in vivo the following procedure was used. Blood was taken from a number of rats after 5 days on the caesium diet. The blood was immediately centrifuged and the plasma recovered. Three rats of the same group were used for the membrane potential measurements. These were anaesthetised with sodium pentobarbitone and the muscles to be examined were exposed in turn. In the case of the vastus this was a simple procedure but with the soleus it required some dissection. The tendon of Achilles was cut and the muscles pealed away from the back of the leg exposing the deep surface of the soleus. Special care was taken to avoid drawing blood or interfering with the circulation or innervation of the muscles. On top of each muscle in turn was placed a small perspex bath measuring 10× 15 mm to which was attached a handle held by a micromanipulator. There was an opening in the base of this chamber measuring 3× 10 mm, that is sufficiently large to expose most of the fibres of the underlying muscle. The chamber, pressed firmly on the surface of the muscle, was filled with plasma prepared as described and into this was introduced the silver: silver chloride electrode and tip

of the microcapillary electrode. All parts of exposed muscle not covered by the perspex chamber were covered by parafilm to prevent dehydration. Potentials were measured in about 20 fibres selected at random and the mean value calculated for each muscle. Immediately on completion of the potential measurements the plasma in the bath (about 0.2 ml) was prepared for analysis as was also the muscle. While one soleus and one vastus muscle in each rat was used for the potential measurement, the order in which they were examined was varied. The unused companion muscles and a sample of plasma were also analysed in order to determine whether significant changes in electrolyte content took place during the potential measurements.

Measurement of Net Effluxes of Caesium and Potassium from Phasic and Tonic Muscles of Rat. Rats were given drinking water containing 40 mM CsCl for 2 weeks and were then sacrificed. In the first series of experiments the soleus, extensor digitorum longus (EDL), vastus lateralis and hemidiaphragms were isolated and one of each pair was immediately prepared for analysis while its companion was soaked for 1 h in cold (3—5°) K-free Krebs' fluid containing 10^{-5}/ M ouabain. This solution was not oxygenated but was gently stirred so that conditions could be regarded as hypoxic and unfavourable to the operation of cation active transport. After a period of soaking these muscles were also analysed and net loss of caesium and potassium determined from a comparison of the companion muscles. The difference in cation concentration between each set of companion muscles, that is the apparent efflux of the particular ion, was divided by its mean concentration between freshly dissected and soaked muscles, so that allowance was made for the ionic gradients across the fibre membrane and something approaching a rate coefficient for cation efflux was obtained. In another series of experiments only soleus and vastus lateralis muscles in the caesium-enriched state were used and efflux of the two cations was measured as described above in K-free Krebs' fluid containing 10^{-4} M ouabain bubbled with 5% carbon dioxide in nitrogen. In all experiments the total water content of the muscles and its distribution between the intracellular and interstitial spaces were determined as already described (Dee and Kernan, 1963). Such measurements were required for the calculation of the intracellular concentrations of ions.

In another series of experiments rats were used after the 7th day on the caesium diet. They were anaesthetized with sodium pentobarbitone and one soleus, EDL and vastus lateralis muscle were taken for immediate analysis. About 6 ml of blood was then removed by hypodermic syringe from the carotid artery and this was centrifuged and the plasma separated. Ouabain (10^{-4} M) was added to the plasma and it was bubbled with 5% CO_2 in nitrogen. The companions of the muscles mentioned had been removed while the blood was being centrifuged and these were wrapped in parafilm to prevent drying while the plasma was prepared. They were then soaked for 30 min in the plasma at 15° during continual bubbling with the gas mixture. They were then removed and prepared for analysis. The net loss of potassium and caesium from the soaked muscles was determined by comparison of the companion muscles before and after soaking. The mean membrane potential was also measured in some of these muscles during soaking under these conditions.

Relative Rates of Uptake of Caesium and Potassium by Soleus Muscles in vitro. These were measured by the method of Conway and Moore (1945). Companion muscles with nerve attached were soaked for up to 2 h in oxygenated modified Krebs' fluid at 37°, in which about 100 mM Na had been replaced by an equivalent quantity of either K^+ or Cs^+. The muscles were weighed at 30-min intervals and the rates of increase in weight noted for the 2 bathing fluids. These rates gave an indication of the relative rates of uptake of the cations under investigation. As the muscles used had nerves attached (Dockry, Kernan and Tangney, 1966) special precautions had to be taken to ensure that these were not weighed with the muscles.

The nerves were placed on a platform close to and on a level with the pan of the balance during the weighing of the muscles. Furthermore at the end of a series of weighings each muscle was weighed before and immediately after removal of the nerve from the muscle in order to ensure that the nerve had not introduced an error into the weighing of the latter.

Results

Caesium Accumulation in Blood Plasma and in Soleus and Vastus lateralis Muscles after Various Periods of Exposure of Rats to Drinking Water Containing 40 mM Caesium Chloride. In Fig. 1 are shown the mean changes in blood plasma concentrations of potassium and caesium over a period of 15 days. Here it may be seen that there was an initial rapid increase of caesium concentration of plasma to about 0.35 mEq/l over the first 3 days after which the concentration increased more slowly and almost linearly for the remainder of the period examined. Plasma potassium concentration decreased by over 1 mEq/l during the first 3 days and the pattern of its fall did not reflect the increase in caesium concentration. The decrease in plasma potassium exceeded the rise in caesium concentration and this may have been due to a renal effect of caesium (Relman, 1956).

The changes in the intracellular concentrations of caesium in the soleus and vastus muscles (Fig. 2), particularly within the first 5 days were strikingly different. The rate of increase of $[Cs]_i$ was 2—3 times greater in the soleus than in the vastus during this period. After about the 10th day however the caesium concentration in the vastus approached that of the soleus. When the rats had been drinking the caesium chloride solution for 5 days the concentration of this cation in fibre water divided by its concentration in plasma was less than the same ratio for potassium ions (Fig. 3), so apparently the caesium ions had not yet reached an equilibrium distribution across the fibre membrane. In the soleus on the other hand the concentration gradient for caesium ions across the membrane was more than twice that of potassium ions as early as 3 days after exposure of the rats to caesium, having equaled the potassium gradient in about 12 h. By about the 8th day the concentration gradient for caesium in the vastus muscle fibres was slightly greater than that of potassium. This has already been observed (Kernan, 1969) and has been attributed to the presence of a small number of red fibres (18 %) within the vastus muscles.

In the soleus muscles $[Cs]_i$ began to decline slowly from about the 10th day onwards (Fig. 2) and this coupled with a steady rise in plasma concentration led to a fall in the concentration gradient for caesium across the fibre membrane. This gradient was still about 40 % greater than that of potassium at the end of the 15th day. In the vastus muscles

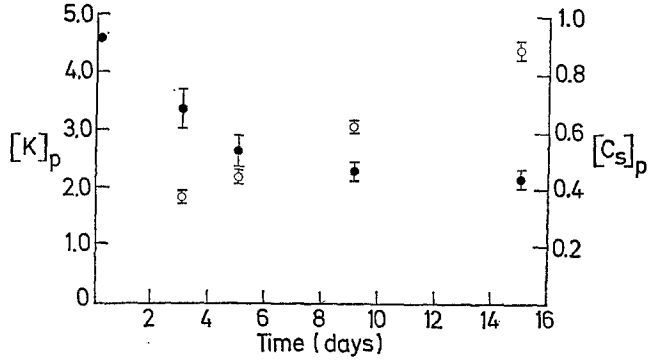

Fig. 1. Changes in potassium (•) and caesium (o) concentrations of blood plasma from rats drinking water containing caesium chloride for various periods of time

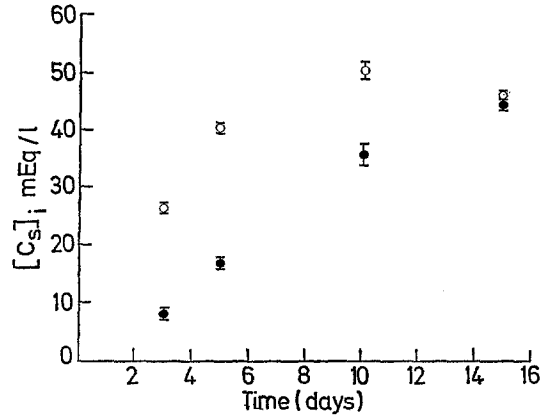

Fig. 2. Increase in caesium concentration of intracellular water of soleus (o) and vastus lateralis (•) muscles of rats given caesium chloride in their drinking water

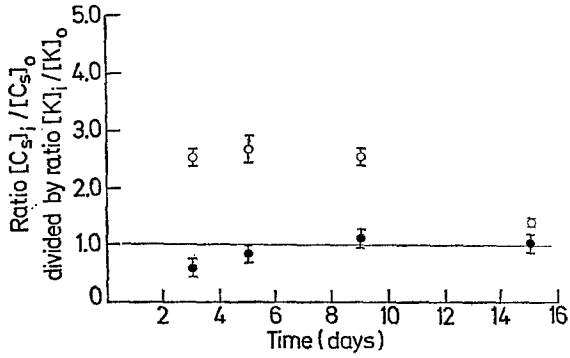

Fig. 3. Changes in the value of the concentration ratio $[Cs]_i/[Cs]_o$ divided by $[K]_i/[K]_o$ in soleus (o) and vastus lateralis (•) muscles during 15 days exposure of rats to drinking water containing caesium chloride

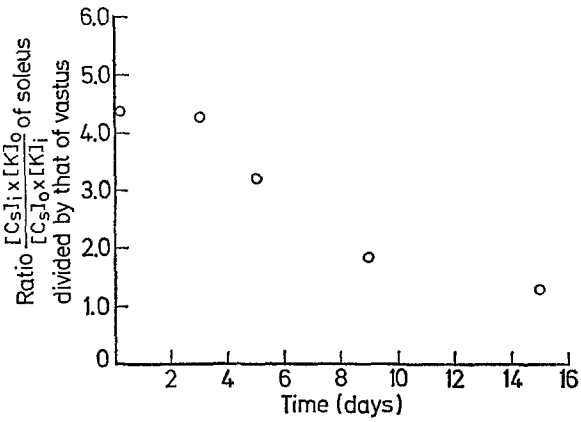

Fig. 4. Concentration ratio $[Cs]_i \times [K]_o / [Cs]_o \times [K]_i$ for soleus muscles divided by the same ratio for vastus lateralis muscles at various time during exposure of rats to drinking water containing caesium chloride

the concentration gradients for potassium and caesium were identical at this time. The difference between soleus and vastus muscles with respect to accumulation of caesium is perhaps best illustrated (Fig. 4) by dividing the ratio of caesium over potassium concentration gradients for soleus muscles by the same ratio for the vastus muscles. This procedure gave a maximum value of about 4.6 on the 3rd day which declined almost exponentially to about 1.7 on the 9th day and then more slowly for the remaining 6 days. The value found 6 h after infusion of caesium acetate into the gut, when $[Cs]_i$ in soleus and vastus muscles were 9.6 ± 0.4 and 2.4 ± 0.2 mEq/l fibre water, respectively, was 4.4. These results indicated that the greatest difference between soleus and vastus muscles with respect to caesium accumulation occurred early after exposure of the muscles to this cation when its influx rate might be expected to exceed its efflux rate.

Membrane Potential Measurements on Soleus and Vastus Muscles in vivo. In Table 1 are shown the mean potentials of soleus and vastus muscles measured in plasma *in vivo* by microelectrode on the 5th day of exposure of the rats to the caesium and also the calculated equilibrium potentials of potassium and caesium in the case of each of the 3 rats used. It is evident from the table that the mean measured potentials were very similar in both muscles but were small in magnitude compared with the equilibrium potentials. This was surprising as it had been previously found (Kernan, 1963) that the mean measured potential was identical with E_K in the case of isolated EDL muscles bathed in rat plasma. To determine whether this discrepancy was due to the presence

Table 1. *Mean resting potentials of rat vastus and soleus muscles measured in vivo and also the calculated equilibrium potentials of potassium and caesium in the same muscles*

	Rat 1	Rat 2	Rat 3	Mean Values
Vastus lateralis				
E_m	-79.0 ± 1.5	-78.0 ± 0.8	-77.5 ± 1.1	-78.0 ± 0.4
E_K	−96.5	−94.0	−90.0	−93.5
E_{Cs}	−107.0	−94.0	−89.5	−96.8
Soleus				
E_m	-86.5 ± 1.3	-80.0 ± 1.4	-75.0 ± 1.2	-79.0 ± 0.4
E_K	−94.0	−90.0	−91.0	−92.0
E_{Cs}	−140.0	−111.0	−110.0	−120.0

Membrane potential in millivolts ± s.e.

All rats had been given caesium solution for 5 days immediately prior to the experiment.

of caesium in plasma and tissues, E_m and E_K were compared in normal rats under the *in vivo* conditions described above and were found to be -82.0 ± 0.2 and -84.0 ± 2.8 mV, respectively (6 EDL muscles in 3 rats used). The difference here was not statistically significant ($P = 0.5$), so it seemed that the replacement of plasma potassium by caesium with consequent lowering of its concentration from about 4.3 to about 3.0 mEq/l plasma water probably contributed to the depolarization of the muscle fibres.

Rates of Potassium and Caesium Efflux from Various Muscles into Anoxic K-free Krebs' Fluid at Low Temperature. The net efflux of potassium from the four muscles examined (Table 2) was greater than that of caesium as one would expect considering the greater intracellular concentration of the former cation. When the net loss of cations from the muscles during 1 h was divided by the mean intracellular concentration of the cations between freshly dissected and soaked muscles in each case a value approaching a rate coefficient was obtained for both the potassium and caesium efflux from the various muscles. In the 4 muscles which had been immersed in cold K-free Krebs' containing ouabain and without bubbling of any kind (Table 3A) the only muscles in which there was a significant difference between rate coefficients for potassium and caesium efflux was diaphragm. Caesium efflux rate here was significantly slower ($P < 0.01$) than that of potassium which was consistent with the tendency of this muscle to accumulate caesium (Kernan, 1969). The value of the ratio k_K/k_{Cs} (Table 3) was indicative of the tendency for caesium to be retained within the fibres during efflux. That is to say, for a similar

Table 2. *Net loss of potassium and caesium from freshly dissected muscles during 1 h soaking in A cold hypoxic potassium-free Krebs' fluid containing 10^{-5} M ouabain, B same fluid containing 10^{-4} M ouabain bubbled with 5% CO_2 in nitrogen*

Muscles	Cation loss (mEq/l F.W. ± s.e.)	
	Potassium	Caesium
A		
Vastus lateralis	18.7 ± 3.3	11.6 ± 1.8
EDL	14.8 ± 4.0	10.1 ± 1.7
Diaphragm	24.2 ± 3.9	9.1 ± 0.8
Soleus	14.4 ± 3.2	10.8 ± 1.8
12 pairs of muscles in each experiment		
B		
Vastus lateralis	25.0 ± 3.8	9.1 ± 1.5
Soleus	8.8 ± 3.5	3.8 ± 1.3
8 pairs of muscles used in each experiment		

Table 3. *Rate Coefficients obtained by dividing net efflux of each cation by its mean intracellular concentration during one hour*

Muscles	k_K	k_{Cs}	k_K/k_{Cs}
A			
Vastus lateralis	0.16 ± 0.05	0.23 ± 0.04	0.68 ± 0.13
EDL	0.19 ± 0.04	0.18 ± 0.04	1.06 ± 0.10
Diaphragm	0.28 ± 0.01	0.17 ± 0.02	1.68 ± 0.02
Soleus	0.15 ± 0.05	0.17 ± 0.03	0.86 ± 0.15
B			
Vastus lateralis	0.29 ± 0.05	0.22 ± 0.05	1.32 ± 0.04
Soleus	0.17 ± 0.04	0.10 ± 0.04	1.70 ± 0.10

intracellular concentration of the two cations in a particular muscle, the greater the value of k_K/k_{Cs} the more potassium in preference to caesium would be exchanged for extracellular sodium. When this ratio was compared therefore in the various muscles the values for soleus and vastus were 0.86.± 0.15 and 0.68 ± 0.13 respectively and the difference between these values was not significant ($0.4 > P > 0.3$). The difference between the values of the ratio for soleus and diaphragm and between that of vastus and diaphragm were both highly significant ($P < 0.01$). These results did not explain the striking accumulation of caesium in soleus muscles nor the fact that such accumulation was greater in the soleus than in the diaphragm (Kernan, 1969). If caesium accumulation was the result of slower efflux rate then the cation should have been more

Table 4. *Permeability coefficients for caesium and potassium efflux from caesium enriched muscles during 30 min at 15° in anoxic plasma containing ouabain to block reaccumulation of these cations*

Muscle	1	2	3	4	5	6	7	8	9
				(3/2)				(7/6)	
	$[Cs]_i$ (mEq/l)	$E_{Cs}-E_m$ (mV)	Cs^+ Efflux (mEq/l)	P_{Cs}	$[K]_i$ (mEq/l)	E_K-E_m (mV)	K^+ Efflux (mMEq/l)	P_K	P_K / P_{Cs}
Soleus	44.0	− 94.5	− 3.0	0.07	80.2	−77.0	− 8.5	0.30	4.4
	(± 0.3)	+ 50.0	(± 0.2)		(± 1.0)	+ 50.0	(± 1.6)		
EDL	40.0	− 92.0	− 4.5	0.16	103.0	− 83.0	− 13.0	0.72	4.5
	(± 0.8)	+ 65.0	(± 0.6)		(±3.0)	+ 65.0	(± 1.5)		
Vastus	26.6	− 82.5	− 7.9	0.31	90.7	− 79.0	− 26.5	1.23	4.0
lateralis	(± 2.8)	+ 57.5	(±1.5)		(± 3.9)	+ 57.5	(± 1.6)		
	Plasma Analysis $[Cs]_o$ 0.9 ± 0.06				$[K]_o$ 3.35 ± 0.21 mEq/l plasma water				

10 pairs of muscles were used in each experiment. Concentrations in muscles expressed as mEq/l fibre water + s.e. of mean.

concentrated in diaphragm which had a k_K/k_{Cs} ratio of 1.68 than in soleus which had a ratio of 0.86.

In the case of the soleus and vastus muscles which had been soaked in cold Krebs' fluid bubbled by nitrogen (Table 3 B) the rate coefficients for caesium efflux was less than that for potassium in both muscles but the difference between these coefficients in each muscle was not significant ($0.4 > P > 0.3$ and $0.3 > P > 0.2$). In this case however the ratio k_K/k_{Cs} differed significantly ($P < 0.01$) in the two muscles and the slower exit of caesium was in the soleus muscles.

Permeability Coefficients for Efflux of Caesium and Potassium Ions from Soleus, EDL and Vastus lateralis Muscles into Anoxic Plasma Containing 10^{-5} M Ouabain at 15°. In this experiment in addition to measuring the net loss of caesium and potassium ions from the muscles by comparison of freshly dissected and soaked companions, membrane potentials were also measured in the muscles during the period of soaking. It was possible therefore to calculate the electrochemical potential gradients producing the loss of cations from the fibres in the muscles examined. The force acting on the caesium ions in each muscle was the difference between the equilibrium potential for this cation, which could be calculated from its mean intracellular and plasma concentrations, and the measured membrane potential. A similar measurement was made of the force acting on the potassium ions in each muscle. When the electrochemical gradient for each ion calculated in this way (Table 4)

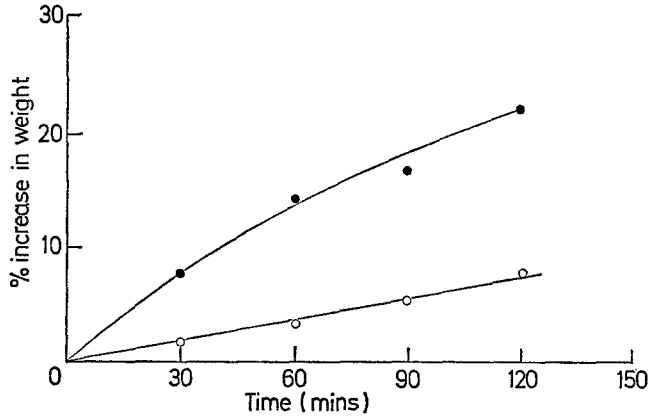

Fig. 5. Relative uptake rates of potassium (•) and caesium (o) into innervated soleus muscles immersed in modified Krebs' fluid at 37° in which 100 mM-NaCl has been replaced by an equivalent of either KCl or CsCl

was divided into the net loss of the particular cation from the fibre water a value for the membrane permeability to the cation was obtained. It may be seen from the table that the permeability coefficient for potassium was greater than that of caesium in each muscle by a factor of about 4 to 1. It was also evident that the permeability coefficient was less for both cations in the soleus muscle than in the vastus lateralis with the EDL muscles having intermediate values. The proportionality of the coefficient in vastus, EDL and soleus muscles respectively was approximately 4:2:1. The average plasma concentration of caesium and potassium during the soaking period are shown at the foot of the Table; the mean concentrations in freshly isolated plasma were 0.67 $\pm$ 0.04 and 2.95 $\pm$ 0.21 mEq/l plasma water for caesium and potassium, respectively. When 16 fibres were examined in each muscle mean membrane potentials were 50.0 $\pm$ 1.5, 65.0 $\pm$ 0.4 and 57.5 $\pm$ 1.3 mV for soleus, EDL and vastus muscles, respectively. The lower potentials in the case of the vastus muscles may have been due to the fact that the whole muscle was not used in the experiment but rather a bundle of fibres of about the same total diameter as the soleus and EDL muscles. Although the fibres appeared to be intact from end to end there may have been some slight damage to them during dissection. It was found in this experiment that the permeability ratio P_K/P_{Cs} for cation efflux was very similar in all 3 muscles in spite of the variability of the individual coefficients from one muscle to the next. This would suggest that caesium accumulation in the soleus muscles is not due to slower efflux relative to potassium as a similar slow efflux compared to that of potassium also exist in vastus which exhibits no caesium accumulation.

Relative Rates of Caesium and Potassium Uptake in Soleus Muscles Soaked in Modified Krebs' Fluid. When the relative rates of uptake of caesium and potassium were measured from the rates of swelling of muscles in modified Krebs' solution containing 100 mM Cs and 100 mM K respectively in place of sodium, it was found (Fig. 5) that the former cation was taken up at about one fourth the rate of the latter over a period of 1 h at 37°. Potassium however appeared to be accumulated more slowly in comparison to caesium in the soleus than in the pale fibres of frog sartorius (Conway and Moore, 1945).

Discussion

The most striking difference between soleus and vastus muscles with respect to caesium accumulation occurred within the first three days on which the rats were given caesium in their diet. During the first four to five days the distribution of caesium ions between the muscle fibre water and plasma in the case of the vastus had not reached equilibrium with the potassium distribution and this fact was in keeping with the reported low permeability of pale muscles to caesium (Conway and Moore, 1945; Bolingbroke *et al.*, 1961). Boyle and Conway (1941) were the first to suggest that potassium and chloride ions became distributed across the muscle fibre membrane in a Donnan equilibrium. This view was tested and confirmed by them in experiments with frog sartorius muscles. It was subsequently confirmed also by Hodgkin and Horowicz (1959) in experiments employing the microelectrode technique. As the caesium in the rat vastus muscles eventually approached a distribution between muscle fibre water and plasma which was similar to that of the potassium ions it seemed likely that these also became passively distributed in a Donnan equilibrium across the fibre membrane. However since the mean value of the measured potential (Table 1) in the vastus was less negative than E_K and E_{Cs} passive uptake of these cations would seem unlikely unless part of the intracellular cations were bound, for example in mitochondria. Depolarization of the muscles in presence of caesium was probably due to a decrease in the P_K/P_{Na} ratio as applied to the Goldman equation (Adrian, 1956). Steady state conditions existed during the electrical measurements as there was no change in the composition of the plasma bathing the muscles or of the muscles themselves. Caesium has been found to inhibit potassium exchange in muscle (Adrian, 1964; Bolingbroke *et al.*, 1961; Sjodin, 1959) so a decreased permeability of the membrane to potassium may have produced a reduced P_K/P_{Na} ratio in our muscles.

In the soleus muscles the concentration gradient for caesium between the inside and outside of the fibre membrane was already in excess of

that of potassium within 24 h of giving caesium solution to the rats and was about 2.5 times the latter on the 3rd day. The resting blood flow of soleus muscles was found to be 3—4 times greater than that of gastrocnemius (Hilton and Vrbova, 1968) and possibly greater than that of other pale phasic muscles. While this might facilitate caesium accumulation to some extent it seems unlikely that it would account for the striking difference in the relationship of the concentration gradients of caesium and potassium in the soleus and vastus lateralis muscles (Figs. 3 and 4). The accumulation of caesium in the soleus must be attributed either to its active transport into the fibres or to its selective binding within the cell. The latter mechanism would seem to be supported by the observations of Olsson, Söremark and Wing (1969) and of Nelson and his colleagues (1961). The former workers used ^{86}Rb and ^{43}K in autoradiographic studies and found that the former was selectively taken up in hyaline, articular cartilage and tendon in addition to skeletal muscle. The results of Nelson and his associates, who employed ^{137}Cs were in general agreement with these observations that the rare alkaline metal ions were selectively bound. The chemical nature of tendon and related tissues would appear to favour adsorption such as found in an ion exchanger. However since we removed all visible tendon from our muscles before analysis it seems unlikely that this was the site of selective accumulation in the soleus muscles. The likelihood of selective adsorption of caesium in soleus muscles accounting for its accumulation also seems remote for the following reason. When rats had ingested caesium to the point where 40 % of the intracellular potassium had been replaced by this action, the sum of $[K]_i + [Cs]_i$ was found to be 155.0 ± 2.6 mEq/l fibre water compared with 153.0 ± 1.5 mEq/l for intracellular potassium concentration in freshly dissected muscles of normal untreated rats. The mean water content of the muscles were 76.3 ± 0.11 and 76.4 ± 0.9 ml/100 g wet weight of tissue, respectively. This would seem the rule out selective binding of caesium as it was contributing as effectively as potassium ions to the osmotic activity of the cell. The most likely reason for the striking accumulation of caesium within the red muscle fibres would seem to be that it was actively transported into the fibres rather than taken up through permeability channels towards a Donnan equilibrium. The ion carrier mechanism in the membrane which normally transports potassium may show a preference for caesium to the exclusion of potassium in which case it would be unnecessary to refer to relative efflux rates of the two cations in order to explain selective accumulation of caesium in the red fibres.

The observation that the greatest concentration gradient of caesium across the fibre membrane in soleus was established within 3 days while $[Cs]_i$ was still increasing rapidly, with influx rate therefore in excess of

efflux rate, coupled with the fact that the gradient actually tended to decline as $[Cs]_i$ stabilized, would seem to suggest that a difference in influx rather than efflux rates was responsible for caesium accumulation. This view was also supported by the similarity in the k_K/k_{Cs} ratios for the soleus and vastus muscles found in unbubbled hypoxic fluid (Table 3 A). When the vastus and soleus muscles were soaked for an hour in K-free solution containing ouabain and bubbled with nitrogen to provide strictly anaerobic conditions unfavourable to the operation of the sodium-pump, it was unlikely that ions which had leaked out of the fibres could be reaccumulated. In this case the k_K/k_{Cs} ratio was greater in the soleus than in the vastus muscles (Table 3 B) indicating that differences in efflux rates might contribute to caesium accumulation. However this result was in conflict with results shown in Table 4 where permeability coefficients rather than rate coefficients were compared. In the calculation of permeability coefficients the total electrochemical potential gradient acting on the ions was taken into account rather than concentration gradients alone, therefore the results in Table 4 would seem to be more valid. The permeability coefficients were also measured under conditions more closely approaching to those pertaining to the *in vivo* state. This is perhaps most important where external potassium concentration is concerned in view of the phenomenon of anomalous rectification in muscles (Katz, 1949).

The finding that the permeability coefficient for potassium efflux from the vastus muscle was about 4 times that of the soleus was in keeping with evidence already obtained (Kernan, 1969) of lower potassium permeability in the soleus compared with EDL muscles. Although there is some uncertainty about the relative surface to volume ratio in fibres of the muscles under discussion, it seems unlikely that this alone would be sufficient to account for a four-fold difference in the apparent permeability of the fibre membrane.

The hypothesis has been advanced that the uptake of potassium and of caesium might be mainly through permeability channels in the pale muscle fibres and mainly via the pump in red fibres and that caesium accumulation in the later might be due to a greater affinity of the membrane carrier molecule for this cation relative to potassium. Such a difference would imply different coupling ratios for sodium-potassium exchange on the cation pump in these muscle types. If the potassium concentration gradient across the fibre membrane in the steady-state is determined by the membrane potential and if the latter is determined in turn by the activity of the sodium pump (Akiyama and Grundfest, 1971), then the greater Na/K exchange ratio proposed here for the pale muscle would be consistent with the more negative membrane potential (Harris and Luff, 1970) and greater concentration gradient for potassium (Dra-

hota, 1960; Sreter and Woo, 1963) across the fibre membrane of pale as compared with red fibres. The higher membrane resistance of slow fibres compared with twitch fibres (Adrian and Peachey, 1965) would also be consistent with a lower permeability of the latter muscles to potassium.

When the uptake of caesium and potassium in soleus muscles was measured under conditions favouring influx through permeability channels (Fig. 5) the former cation appeared to be taken more slowly than the latter.

The main conclusions of the present paper are that it is the difference in influx rates rather than in efflux rates of the caesium and potassium ions which determines the striking accumulation of caesium in the red fibres of soleus and the absence of a similar uptake in pale fibres of the vastus. While it is possible that caesium uptake in the latter muscle is mainly active and carrier mediated it seems unlikely that the similarity of the concentration gradients for potassium and caesium across the fibre membrane, which suggests passive uptake, is purely fortuitous.

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