J. Physiol. (1965), **177**, *pp.* 453–462
With 6 text-figures
Printed in Great Britain

POTASSIUM AND RUBIDIUM EXCHANGE ACROSS THE SURFACE MEMBRANE OF CARDIAC PURKINJE FIBRES

BY P. MÜLLER

From the Department of Physiology, University of Berne, Berne, Switzerland

(*Received* 18 *September* 1964)

When Sidney Ringer in 1883 reported on the ionic requirements of the isolated frog heart he mentioned: 'We find then that rubidium can largely replace potassium'. Little further information was subsequently collected with respect to the fate of Rb in cardiac tissue. The results obtained with other types of cells (nerve, skeletal muscle) indicate that K and Rb are treated in a similar but not in exactly the same way. As a rule, the permeabilities of the cell membrane to K and Rb are not equal; competition for uptake has been reported; and muscle cells have been shown to accumulate Rb in preference to K.

Results of electrical measurements and data on ^{42}K and ^{86}Rb flux will be compared to see which effects the two ions have in common, and in which respects they differ. Some of the present results have been reported in a preliminary communication (Müller, 1961).

METHODS

The membrane potential and the membrane resistance of single Purkinje fibres of sheep and calf were measured using Ling-Gerard (1949) micro-electrodes as described by Weidmann (1956). The preparations, which as a rule showed spontaneous activity, were stimulated by means of external electrodes at a rate of 1/sec. The tissue bath had a capacity of 0·3 ml. A corresponding volume of Tyrode solution was made to flow past the preparation in 2–5 sec.

The K- and Rb-rich test solutions were prepared by adding solid KCl or RbCl to K-free Tyrode solution; the Na content was not changed. Sodium-deficient solutions were prepared by substituting choline chloride for NaCl. Atropine sulphate (20 mg/l.) was added to avoid ACh-like effects. ^{42}KCl and $^{86}RbCl$ solutions were obtained from spectroscopically pure $^{42}K_2CO_3$ and $^{86}Rb_2CO_3$ (Eidgenössisches Institut für Reaktorforschung) by titration with 0·1 N-HCl to pH 7·0. Adding different molar concentrations of ^{42}KCl and $^{86}RbCl$ provided the radioactive test solutions. The radioactivity of the preparations and the bathing solutions was measured in a well-type crystal SC-46 and a Tracerlab Scintillation detector P-20 C. A test-tube method as described by Carmeliet (1961) was used in all the uptake and efflux experiments; the preparations were not stimulated. For calibration purposes known amounts of the charging solutions were melted into polyethylene tubes and counted under the same conditions as the preparations, with 5 ml. of fluid surrounding the source of radioactivity.

RESULTS

The effect of K and Rb on the membrane potential

When RbCl was added to a Tyrode solution completely free of potassium the preparations were irreversibly damaged at the end of the first hour. Short-term exposures to Rb alone, or long-lasting exposures to Rb-K mixtures resulted in reversible changes.

Substituting 2·7 mM-RbCl for 2·7 mM-KCl had no significant effect on the value of the so-called 'maximum diastolic potential' nor on the shape and amplitude of the action potential. As expected, increasing the extracellular potassium concentration, $[K]_o$, decreased the resting potential (Fig. 1). Over a large concentration range, 8·1–54 mM, rubidium was somewhat less effective in depolarizing the membrane than potassium (Figs. 1, 2). This may be taken to suggest that the permeability to rubidium, P_{Rb}, is lower but comparable to the permeability to potassium.

There is reason to assume that at high $[K]_o$ values the membrane potential is close to the potassium equilibrium potential, where K influx equals K efflux. In Rb solutions the membrane potential would be expected to take such a value that K efflux was equal to Rb influx. For instance, at a potential of 50 mV, $P_K:P_{Rb}$ being say 2:1, a given K efflux could be balanced by the influx either from 13 mM-K Tyrode or from a 26 mM-Rb Tyrode. If the permeability ratio were constant for higher $[Rb]_o$ values (or for lower membrane potentials) the Rb curve should be shifted to the right, parallel to the K curve (Fig. 2). Carmeliet (1961) and Hall, Hutter & Noble (1963) have shown that P_K (as defined by Goldman, 1943) stays substantially constant when $[K]_o$ is increased. The present results may signify that at high $[Rb]_o$ values P_{Rb} drops, possibly by a saturation of extracellular binding sites for Rb (Sjodin, 1961).

Figure 2 shows another unexpected feature: a drop of the membrane potential by some 25 mV for a relatively small increase of $[Rb]_o$ from 13·5 to 16·2 mM. The corresponding curve for depolarization by $[K]_o$ has a steep part in the same region. The usual procedure to obtain the values plotted in Fig. 2 was to depolarize with K or Rb until a steady value was reached (2–4 min), and to let the membrane repolarize in 2·7 mM-K Tyrode (3–10 min) before the next concentration of K or Rb was tested. It was felt that a potential-dependent increase of Na influx might provide an explanation for the steep drop of the membrane potential between 70 and 50 mV. If the membrane conductance for other ions were relatively low the 'jump' should show up especially well. In two successful experiments K and Rb were compared with respect to their effect on total membrane conductance. While there was no measurable difference when changing from 2·7 mM-K to 2·7 mM-Rb Tyrode, the increase of conductance in high Rb solutions was indeed much less pronounced than that observed in high K solutions, the comparison being made at the same membrane potentials. Furthermore, with choline chloride replacing NaCl the 'jump' was considerably less pronounced though not completely absent.

The effect of extracellular Rb on K efflux

When potassium is omitted from the bathing solution Purkinje fibres rapidly depolarize to a new level in the region of 40–50 mV (Weidmann, 1956). The presence of Na ions in the extracellular space is necessary for

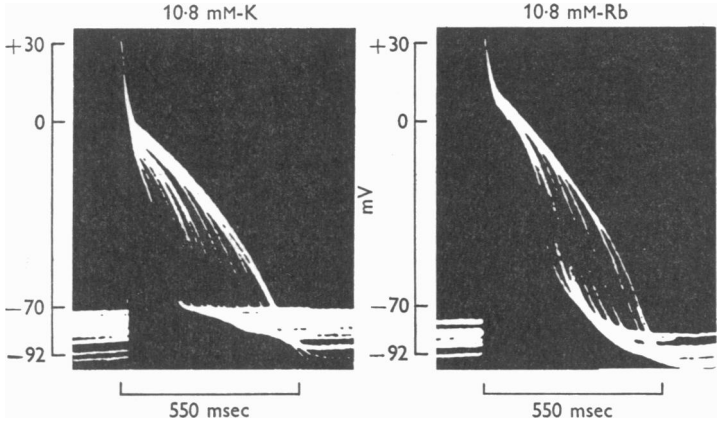

Fig. 1. Effects of equimolar K and Rb concentrations on the membrane resting and action potential. Action potentials were photographed at intervals of 10 sec and superimposed on the same frame. On the left: changing from a 2·7 to a 10·8 mM-KCl Tyrode solution; on the right: changing from 2·7 mM-KCl to 10·8 mM RbCl Tyrode solution. The effects were qualitatively the same; but K was more efficient in depolarizing the resting membrane and in shortening the action potential. Ordinate: membrane potential in mV. Abscissa: time in msec.

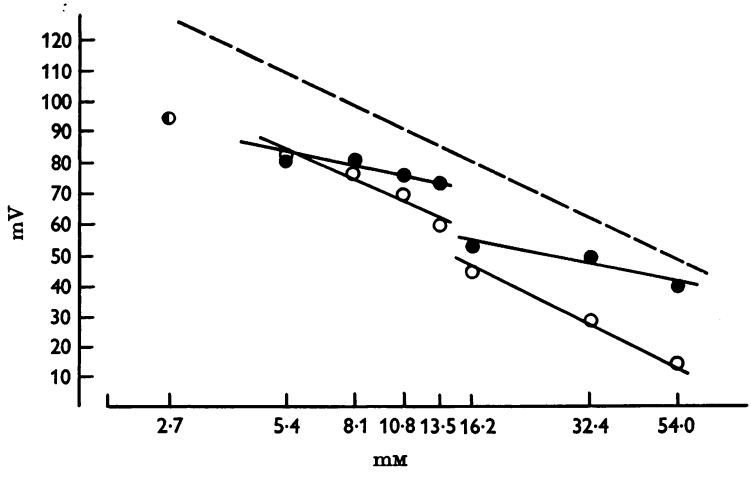

Fig. 2. Effect of $[K]_o$ and $[Rb]_o$ on the membrane potential. Open circles: mean values of resting potentials from 15–20 experiments obtained by increasing $[K]_o$. Closed circles: mean values of the resting potentials obtained by increasing $[Rb]_o$. Standard errors are smaller than diameter of circles. Ordinate: membrane potential in mV. Abscissa: $[K]_o$ and $[Rb]_o$ (log scale). Interrupted line: theoretical slope for a K-electrode, 61·5 mV for a tenfold change of $[K]_o$.

depolarization. ^{42}K efflux is decreased suggesting that P_K is low (Carmeliet, 1961). The assumption is made that in a K-free Tyrode solution the membrane potential reaches a value at which a weak net K efflux is balanced by a weak Na influx (Carmeliet, 1961). Purkinje fibres that have been left in K-free Tyrode for more than an hour can be repolarized within less than a minute if $[K]_o$ is raised to normal. Simultaneously, ^{42}K efflux is increased although the driving force for K efflux gets lower.

It was of interest to see whether or not Rb could replace K with respect to its repolarizing effect. Adding 0·7 and 1·4 mM-RbCl to K-free Tyrode solution repolarized the membrane with a greater regularity than adding equimolar amounts of KCl to the same preparations (30 experiments). This result would indicate that at low concentrations rubidium is even more efficient than potassium in raising the net efflux of electrical charge.

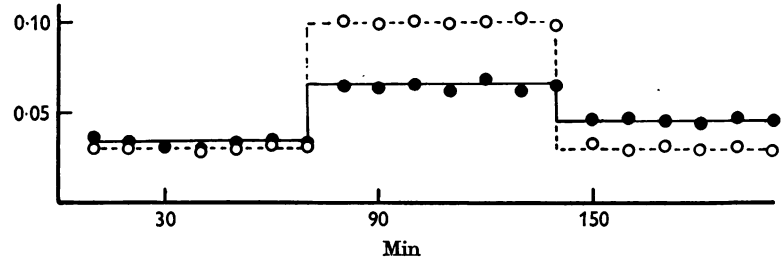

Fig. 3. Effect of K and Rb on ^{42}K efflux. After 5–7 hr uptake periods, the ^{42}K efflux was followed into KCl and KCl plus RbCl solutions. The percentage ^{42}K-loss per minute (ordinate) was plotted against time (abscissa). Open circles: ^{42}K efflux into 0·7 mM-KCl, 5·4 mM-KCl, and again into 0·7 mM-KCl. Closed circles: ^{42}K efflux into 0·7 mM-KCl, 0·7 mM-KCl plus 4·7 mM-RbCl, and again into 0·7 mM-KCl Tyrode solution. Efflux was increased about fivefold by high K, about twofold by high Rb.

The shortening of the action potential in a K-rich solution, as seen in Fig. 1, is explained by saying that a high $[K]_o$ results in a high potassium conductance and a larger K efflux during the plateau of the action potential, when the driving force is in the outward direction (Hall & Noble, 1963). Figure 1 gives an example of the general finding that Rb was *less* efficient in shortening the action potential than K.

For testing the effect of $[K]_o$ and $[Rb]_o$ on the ^{42}K efflux, conditions were chosen under which the membrane potential was reasonably well known: near 50 mV for 0 or 0·7 mM-K; near 80 mV for 5·4 mM-K and 5·4 mM-Rb. By increasing $[K]_o$ or $[Rb]_o$ the outward driving force for ^{42}K could only be lowered; and if the rate of ^{42}K efflux increased—which it did (Fig. 3)—this could only mean that the K permeability increased. The effect was more pronounced for K than for Rb. In five experiments the

factors were from 2 to 3 for K, from 1·5 to 1·8 for Rb. About twice as much ^{42}K efflux was also observed if bathing solutions containing 12·5 or 32·4 mM-K were compared with solutions containing 12·5 or 32·4 mM-Rb (three experiments). The results of flux experiments with ^{42}K are in agreement with the stronger effect of potassium in shortening the action potential.

The effect of K on the ^{86}Rb efflux

The finding that Rb increased the K efflux suggested the possibility that K might increase the Rb efflux. Because of the large difference in half life (12 hr and 18·7 days) it was possible to measure ^{42}K and ^{86}Rb efflux simultaneously. In the experiment illustrated by Fig. 4 $[Rb]_o$ was kept constant at 2·7 mM throughout an efflux experiment while $[K]_o$ was varied from 2·7 mM to zero, and again to 2·7 mM. As expected there was an influence of $[K]_o$ on the ^{42}K efflux; but there was practically none on the ^{86}Rb efflux.

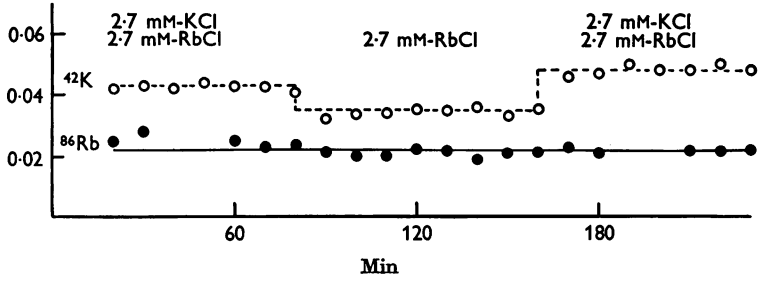

Fig. 4. Effect of $[K]_o$ on the ^{42}K and ^{86}Rb efflux. The preparation had been loaded in a 2·7 mM-^{42}KCl plus 2·7 mM-$^{86}RbCl$ solution for 5 hr. The percentage loss per minute of the two cationic species is plotted against time. Extracellular potassium had a definite effect on ^{42}K efflux but no clear-cut effect on ^{86}Rb efflux.

Two experiments of this kind were done with charging periods of 5 and 7 hr respectively in 2·7 mM-^{42}K plus 2·7 mM-^{86}Rb preceding the efflux measurements. It will be shown below that the charging time was not quite sufficient for Rb to reach an equilibrium distribution. For this reason the difference in the efflux rate constants (Fig. 4) must be slightly overestimated.

The effect of Rb on the ^{42}K influx

Potassium influx into Purkinje fibres rises when $[K]_o$ is increased; but the rise is less than proportional to the increase of $[K]_o$ (Carmeliet, 1961). For instance, when $[K]_o$ was increased tenfold, from 0·54 to 5·4 mM, K influx went up only fourfold (Carmeliet). This would be expected if K ions competed for a transport system having a limited capacity. In the experiment illustrated by Fig. 5 two solutions were compared which had the same

^{42}K concentration but were supplemented either by K or Rb in equimolar amounts. When looking at the middle step of Fig. 5 it must be realized that ^{42}K influx was slowed down by the presence of inactive potassium; it is evident from the first and third step that rubidium was even more efficient than potassium in slowing down ^{42}K influx. The same observation was made with frog skeletal muscle (Sjodin, 1961).

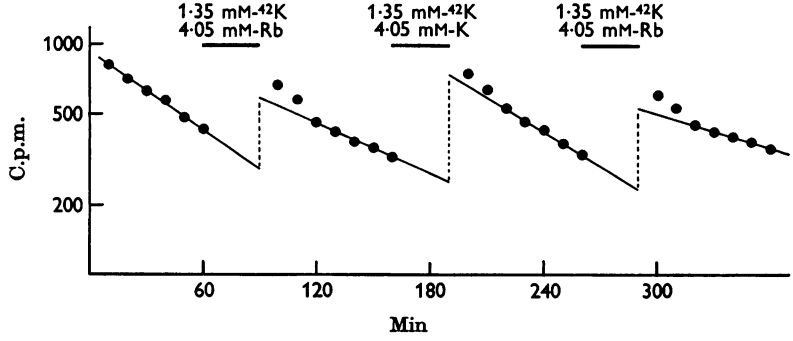

Fig. 5. Effect of Rb on ^{42}K influx. The radioactivity of the preparation was followed as a function of time. The tissue was exposed to ^{42}K solution for periods of 30 min, 3 times in the course of this experiment. By extrapolating the efflux curves through the charging periods a correction was made for the ^{42}K loss during ^{42}K uptake. The radio-inactive solution contained 5·4 mM-KCl and no RbCl. The lengths of the broken upstrokes provide a measure for ^{42}K inflow. This was lower from a ^{42}KCl-RbCl solution (first and third step) than from an equimolar ^{42}KCl-KCl solution (middle step).

Steady state accumulation of K and Rb

If the two ions were treated in the same way their intracellular concentration after a sufficiently long equilibration period should be equal. Twelve preparations were loaded in 5·4 mM-^{42}KCl plus 5·4 mM-^{86}RbCl. At the end of a 10 hr charging period the preparations were counted for total radioactivity and subsequently, after 5–12 days, for ^{86}Rb alone. A comparison with the charging solutions gave a Rb content roughly twice as large as the K content. In single experiments Rb:K varied between 2·4:1 and 1·7:1. Counts taken after 2 years indicate that there had been no ^{134}Cs contamination in the ^{86}RbCl.

Another way to demonstrate preferential Rb accumulation is illustrated in Fig. 6. In this instance the total activity of a preparation was followed as a function of time. Equimolar concentrations of K and Rb were present in the bathing solution; and the values in Fig. 6 were corrected for the differences in the counting rate of the two radioactive components. The heights of the two steps are therefore proportional to the

steady-state Rb and K contents of the preparations. Again as in two more experiments of this kind, a ratio of about 2:1 was found. The time for half-exchange was larger for Rb (120 min) than for K (80 min). Since the intracellular Rb fraction was larger than the K fraction by a factor of 2, the ratio of the absolute fluxes as measured in pM cm^{-2} sec^{-1} would be close to unity. Assuming $[Rb]_i:[K]_i$ is 2:1 one can calculate roughly a permeability ratio for this special case (Keynes, 1954): $P_{Rb}:P_K = 2:3$.

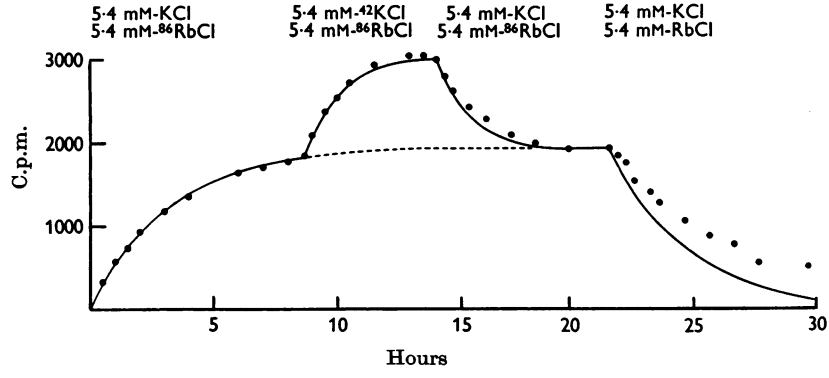

Fig. 6. Simultaneous ^{86}Rb and ^{42}K influx and efflux measurements. At zero time the preparation had been kept in 5·4 mM-K plus 5·4 mM-Rb for 3 hr. The chemical composition of the bathing solution was kept constant throughout the experiment. ^{86}Rb was first substituted for inactive Rb. When equilibrium was approached (^{86}Rb influx = ^{86}Rb efflux) ^{42}K was substituted for inactive K, leaving $^{86}[Rb]_o$ constant. The half time for the Rb exchange was of the order of 120 min that of the K exchange of the order of 80 min. Each filled circle in the graph represents the activity of the preparation after a 5 min washout period of the extracellular space. The experimental values for both influx and efflux were fitted by exponentials.

DISCUSSION

The picture that emerges from the present results is far from simple. While Rb has several effects in common with K, there are, on the other hand, complicated interactions between the two ions. Extracellular Rb decreases the K uptake and increases K release; extracellular K has little if any effect on the Rb efflux. It follows that under steady-state conditions Rb must be accumulated from an equimolar K-Rb solution to a larger extent than K. Preferential accumulation of Rb with respect to K has been demonstrated for skeletal muscle (Lubin & Schneider, 1957; Relman Lambic, Burrows & Roy, 1957). Rb might be stored in an electrochemically inactive form. If so, the sum of the cations, Rb+K, should increase in the course of exposure to Rb-Ringer's solution. It was recently demonstrated with frog skeletal muscle (Adrian, 1964) that $[Rb]_i+[K]_i$

stays constant when Rb replaces intracellular K. This argues against preferential binding to cell constituents being responsible for Rb accumulation.

The tacit assumption has been made that K, as well as Rb, is not subject to purely passive distribution governed by the electrochemical gradient, or more specifically, that there is an 'active' component of K as well as of Rb influx. Otherwise it would not be possible to account for the fact that $[Rb]_o$ has opposite effects on the K influx and efflux.

With respect to the $P_{Rb}:P_K$ ratio, differences exist amongst biological membranes. Crab nerves are depolarized more strongly by Rb than by K ions; also, Rb ions are more effective in raising the membrane conductance (Wilbrandt, 1937; Hodgkin, 1947). These findings are taken to indicate that $P_{Rb} > P_K$. With frog skeletal muscle and mammalian Purkinje fibres Rb ions depolarize to a smaller extent than K ions (Sjodin, 1959; present paper), suggesting that $P_{Rb} < P_K$. The value of $P_{Rb}:P_K$ for Purkinje fibres is clearly concentration dependent (Fig. 2) so that no generally valid figure can be given. Also, it would hardly be justifiable to derive a meaningful $P_K:P_{Rb}$ ratio from experiments like that of Fig. 6. For it must be realized that under the conditions of that experiment Rb was enhancing ^{42}K efflux while K had no major effect on Rb efflux.

It was pointed out to me by Prof. W. Wilbrandt, of Berne, that several of the present findings can be accounted for if it is assumed that K and Rb cross the membrane in combination with the same carrier molecules (see Rosenberg & Wilbrandt, 1963). To explain why Rb is more efficient than K in depressing ^{42}K-influx it is sufficient to postulate a higher carrier affinity for Rb. A slower penetration of Rb in spite of a higher carrier affinity is possible if the carrier-Rb complex has a much lower apparent mobility than the carrier-K complex. Preferential accumulation of Rb within the cell will be expected if by some process depending on metabolic energy the carrier molecule for which Rb has a higher affinity than K were given a concentration gradient from outside inwards (eqn. 16 of Rosenberg & Wilbrandt). This is in keeping with the statement that 'active transport' has to be postulated in order to account for preferential accumulation of Rb. Finally, the low rate of K efflux into a K-free medium may be understood if 'free' carriers have a lower mobility than 'occupied' carriers. Under such conditions carrier molecules accumulate near the outer membrane surface while there is a lack of carriers at the inner surface. Addition of either K or Rb to the bathing solution will bring carriers back to the inside and thereby increase ^{42}K efflux.

The carrier concept seems to offer no explanation for the shortening of the action potential resulting from an increase of $[K]_o$ or $[Rb]_o$. To obtain a more rapid repolarization an increase of the *net* efflux of positive charge

is required. If K ions have first to enter the cell before other K ions can then leave it, we are still without an explanation for the increase of net efflux resulting from an addition of K or Rb to the extracellular space.

SUMMARY

1. With Purkinje fibres from calf or sheep hearts the resting and action potentials did not change when extracellular K (2·7 mM) was replaced by Rb (2·7 mM).

2. Increasing $[K]_o$ or $[Rb]_o$ resulted in a drop of the resting potential; Rb was less effective than K, suggesting $P_{Rb} < P_K$ at relatively high concentrations (8–54 mM).

3. At $[K]_o$ values below 2·7 mM-P_K of the membrane had been reported to be decreased. Evidence was obtained to show that Rb can substitute for K in increasing P_K, thus (i) ^{42}K-efflux was increased by adding Rb-ions to a low-$[K]_o$ Tyrode; (ii) the action potentials were shortened by a rise of $[Rb]_o$ which is in agreement with a rise of K-efflux; and (iii) when a membrane had been depolarized to 40–50 mV in a K-free solution the addition of 1·4 mM-RbCl brought the potential back above 90 mV in less than 1 min.

4. Addition of Rb decreased the rate of ^{42}K-uptake even more than addition of an equimolar amount of K.

5. From a 5·4 mM-K and 5·4 mM-Rb solution the cells accumulated Rb in preference to K (2:1). The intracellular ^{86}Rb was exchanged more slowly than the intracellular ^{42}K, $\tau_{\frac{1}{2}}$ being 120 and 80 min, respectively.

6. Since there is competition for uptake and an effect of extracellular Rb on P_K the independence principle does not hold true for K and Rb movements. The unequal accumulation of the two ionic species prevents the use of rubidium as a tracer for potassium.

My thanks are due to Prof. S. Weidmann, for much helpful discussion, and to Dr H. A. Fozzard, for reading the manuscript. Financial support was provided by the 'Stiftung für biologisch-medizinische Stipendien', the Josephine Clark-Joller Foundation, and the Swiss National Science Foundation.

REFERENCES

ADRIAN, R. H. (1964). The rubidium and potassium permeability of frog muscle membrane. *J. Physiol.* **175**, 134–159.

CARMELIET, E. E. (1961). *Chloride and potassium permeability in cardiac Purkinje fibres.* Bruxelles: Presses académiques Européennes.

GOLDMAN, D. E. (1943). Potential, impedance, and rectification in membranes. *J. gen. Physiol.* **27**, 37–60.

HALL, A. E., HUTTER, O. F. & NOBLE, D. (1963). Current-voltage relations of Purkinje fibres in sodium-deficient solutions. *J. Physiol.* **166**, 225–240.

HALL, A. E. & NOBLE, D. (1963). The effect of potassium on the repolarizing current in cardiac muscle. *J. Physiol.* **167**, 53–54*P*.

HODGKIN, A. L. (1947). The effect of potassium on the surface membrane of an isolated axon. *J. Physiol.* **106**, 319–340.

KEYNES, R. D. (1954). The ionic fluxes in frog muscle. *Proc. Roy. Soc.* B, **142**, 359–382.

LING, G. & GERARD, R. W. (1949). The normal membrane potential of frog sartorial fibers. *J. cell. comp. Physiol.* **34**, 383–396.

LUBIN, M. & SCHNEIDER, P. B. (1957). The exchange of potassium for caesium and rubidium in frog muscle. *J. Physiol.* **138**, 140–155.

MÜLLER, P. (1961). Kalium- und Rubidium-Wirkungen am Herzmuskel. *Helv. physiol. pharmacol. Acta*, **19**, C 91.

RELMAN, A. S., LAMBIC, A. T., BURROWS, A. & ROY, A. M. (1957). Cation accumulation by muscle tissue: The displacement of potassium by rubidium and caesium in the living animal. *J. clin. Invest.* **36**, 1249–1256.

RINGER, S. (1883). An investigation regarding the action of rubidium and caesium salts compared with the action of potassium salts on the ventricle of the frog's heart. *J. Physiol.* **4**, 370–378.

ROSENBERG, T. & WILBRANDT, W. (1963). Carrier transport uphill. *J. theoret. Biol.* **5**, 288–305.

SJODIN, R. A. (1959). Rubidium and caesium fluxes in muscle as related to the membrane potential. *J. gen. Physiol.* **42**, 983–1003.

SJODIN, R. A. (1961). Some cation interactions in muscle. *J. gen. Physiol.* **44**, 929–962.

WEIDMANN, S. (1956). *Elektrophysiologie der Herzmuskelfaser.* Bern, Stuttgart: Huber.

WILBRANDT, W. (1937). The effect of organic ions on the membrane potential of nerve. *J. gen. Physiol.* **20**, 519–541.