Potassium Fluxes in Desheathed Frog Sciatic Nerve

WILLIAM P. HURLBUT

From The Rockefeller Institute, New York

ABSTRACT Desheathed frog (*R. pipiens*) sciatic nerves were soaked in Na-deficient solutions, and measurements were made of their Na and K contents and of the movements of K^{42}. When a nerve is in Ringer's solution, the Na fluxes are equal to the K fluxes, and about 75 per cent of the K influx is due to active transport. The Na content and the Na efflux are linearly related to the Na concentration of the bathing solution, while the K content and the K fluxes are not so related. When a nerve is in a solution in which 75 per cent of the NaCl has been replaced by choline chloride or sucrose, the active K influx exceeds the active Na efflux, and the K content is maintained. When a nerve is soaked in a solution that contains Li, the K^{42} uptake is inhibited, and the nerve loses K and gains Li. When a Li-loaded nerve recovers in a Li-free solution, K is taken up in exchange for Li. This uptake of K requires Na in the external solution. It is concluded that the active transports of K and of Na may be due to different processes, that an accumulation of K occurs only in exchange for an intracellular cation, which need not be Na, and that Na plays a specific, but unknown, role in K transport.

INTRODUCTION

The efflux of Na from frog sciatic nerves is nearly independent of the K concentration of the bathing solution (7). This suggests that in a nerve in standard Ringer's solution no coupling exists between the active Na efflux and the active K influx. To prove the independence of the active transport processes for Na and K, one must demonstrate also that the active K influx is not directly dependent on the active Na efflux. This report presents results indicating that the active K influx is not stoichiometrically related to the active Na efflux. It is concluded that the active transports of Na and K may be brought about by independent mechanisms.

METHOD AND MATERIALS

The method of preparing the nerves and the analytical techniques used in this investigation were the same as those reported in a previous paper (7). The choline content of a nerve was determined with choline labeled with C^{14} in the methyl position.

Preparation of Isotope Solution 2 mc of K^{42} in HCl were diluted to 5.0 ml with water and adjusted to pH 6–8 with NaOH. Appropriate aliquots of this stock solution were added to the physiological fluids to give convenient levels of radioactivity. The stock solution contained K at concentrations of 30 to 60 mM, and this source of K was allowed for when making up the experimental solutions. Except in some solutions used for loading prior to efflux measurements, the concentrations of K in the experimental radioactive solutions fell within ±0.1 mM of the standard concentration of 2.0 mM.

Uptake of K^{42} Usually, each nerve was soaked in 5 to 10 ml of a solution that contained K^{42} at an initial concentration of about 0.2 μc/ml. To determine the influx of K^{42}, the nerves were exposed to radioactive solutions for 0.5 to 2 hours, usually for 1 hour.

When the Na contents of the nerves were not determined, the extracellular K^{42} was calculated from the equation:

$$q^*_{ecs} = ac^*_o[W_T - D - W_i]/D \tag{1}$$

where q^*_{ecs} = the K^{42} content of the extracellular space in CPM/gm (dry)
a = ml of solution per gram water, assumed to be equal to 1
c^*_o = the concentration of K^{42} in the bathing solution in CPM/ml
W_T = the wet weight of the nerve in grams
D = the dry weight of the nerve in grams
W_i = the intracellular water in grams.

The intracellular K^{42} content, Q^*_i, expressed in units of μmols/gm, was calculated from the formula:

$$Q^*_i = (q^*_T - q^*_{ecs})c_o/c^*_o \tag{2}$$

where: q^*_T = the total K^{42} in the nerves in CPM/gm (dry)
c_o = the K concentration of the bathing solution in μmols/ml.

This is the K^{42} content that would have been attained if all the K in the external solution had been replaced with K^{42}.

Previous results (6) indicated that the ratio, W_i/D, was relatively constant and equal to 1.1, approximately, and they showed that most of the variations in the water contents of desheathed nerves were due to variations in the sizes of the extracellular spaces. Therefore, when equation (1) was used, W_i/D was assumed to be 1. After a nerve has been soaked for 1 hour in a solution that contains K^{42}, the extracellular space contains about 25 per cent of the total K^{42} in the nerve. Consequently, the value adopted for W_i/D in equation (1) is not critical.

When the Na contents of the nerves were measured, the nerves were washed for 1 hour at 2°C in a solution that contained 118 mM choline chloride and 1.8 mM $CaCl_2$ (solution W) before they were extracted for analysis. When this was done, it was assumed that no K^{42} was present in the extracellular space. The estimates of intracellular K^{42} obtained using equations (1) and (2) agreed with the estimates obtained from washed nerves.

Efflux Experiments Nerves were soaked for 3 to 4 hours at 20°C in Ringer's solution that contained K^{42} at an initial concentration of approximately 2 μc/ml. In some cases, the K concentration of the loading solution was as high as 6 mM, because of the extra K that was added with the radioisotope. The high concentration of K had no obvious effects. In the early efflux experiments, the radioactive nerves were placed in a capillary arranged so that the eluting solution flowed past the central section of the nerve only, thereby minimizing the effects of the cut ends. In later experiments, the entire preparation was passed through planchets, as was done in the Na^{22} efflux studies (7). These techniques gave similar results.

Extraction Methods Because of the short half-life of K^{42}, it was necessary to extract the nerves rapidly. In the early efflux and uptake experiments, this was done by leaching the nerves for 15 minutes at 100°C in 1.0 ml of 2 M HNO_3. The extract was diluted to 6.0 ml with water, and an aliquot was dried and counted. If the extract was not diluted, a scum formed in the planchet and interfered with the counting. In later efflux experiments, the nerves were extracted for 15 minutes in water at 100°C, as described previously for Na^{22} (7). In the uptake experiments in which K, Na, and K^{42} were determined, the nerves were extracted overnight in water in an oven at 80°C. This procedure removed 98 per cent of the isotope from the nerves (this was determined for Rb^{86} and Cs^{134}), and gave values for the K contents similar to those obtained for nerves that had been leached in water for several days at room temperature.

Unless indicated otherwise, all experiments were conducted at 20°C. The numerical values given in the text are mean values ± 1 SD. The results of all analyses have been referred to the dry weights of the nerves.

RESULTS

Solutions Containing Na at Concentrations near 116 mM. Fig. 1 shows the time course of the uptake of K^{42} by nerves soaked in radioactive Ringer's solution at 2 or 20°C. These nerves were not washed in solution W, and no corrections were made for the K^{42} contained in the extracellular spaces. The values of the K contents of these nerves are listed in Table I. No significant differences in K contents were noted between the nerves that had been soaked at 20°C and those that had been soaked at 2°C. These nerves were extracted in HNO_3, and the mean K content of relatively fresh nerves was 196 μmols/gm, about 40 μmols/gm greater than the K contents of nerves extracted in water (6, 7). When the K content, as given by the acid extraction, was used to compute the specific activity of the K in the nerve, the results indicated that not all of the K was exchangeable. Consequently, the data shown in Fig. 1 were computed by assuming that 20 per cent of the K in these nerves could not exchange with K^{42} in the bathing solution. The points in Fig. 1 are fitted with exponential curves. At 20°C, the time constant of the exponential is 10 hours, and at 2°C the time constant is 56 hours. The temperature coefficient

of the K fluxes is two to three times greater than the temperature coefficient of the Na fluxes (7). The time constant for the K^{42} uptake at 20°C is consistent with the K^{42} efflux rate coefficient that is observed in efflux studies (10 to 15

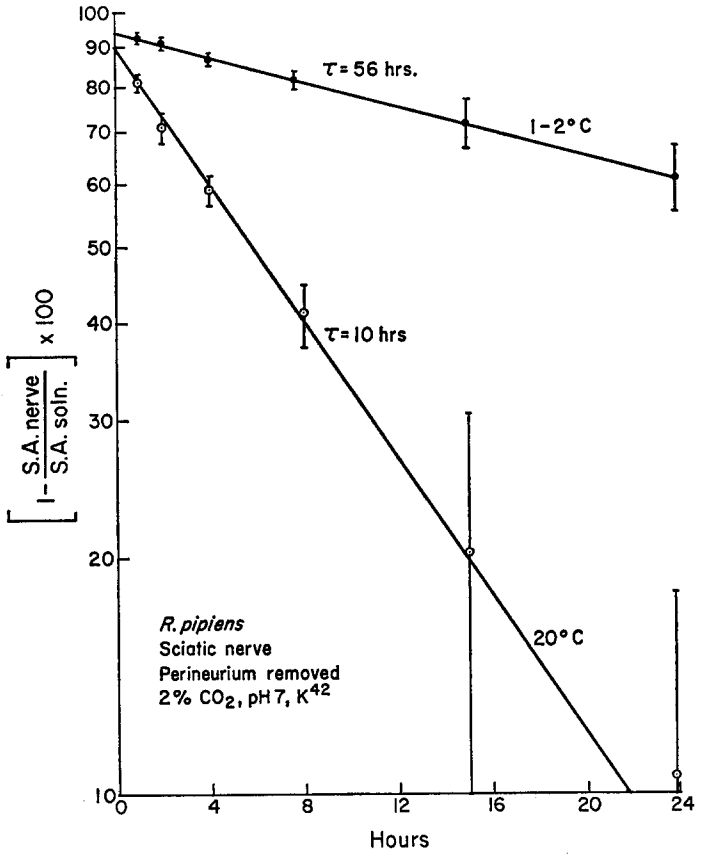

FIGURE 1. Time course of the uptake of K^{42} by desheathed nerves soaked in radioactive Ringer's solution at 20 or at 2°C. The quantity,

$$100 \times \left[1 - \frac{\text{Specific activity of K in nerve}}{\text{Specific activity of K in solution}}\right],$$

is plotted on the ordinate with a logarithmic scale. Abscissa, time in radioactive solution. The specific activity of K in the nerves was computed assuming that 20 per cent of the total K was not exchangeable. The ruled lines are a visual fit to the data. The points are average values based on six to nine determinations, and the vertical bars indicate ± 1 SD.

per cent/hr.). Thus, the exchangeable K in frog nerve is approximately 157 μmols/gm (0.8 × 196), which agrees with the K contents of some 160 μmols/gm determined for water-extracted nerves (6, 7). Apparently, water extraction removes the readily exchangeable fraction of the K in frog sciatic nerves.

About 5 per cent of the exchangeable K (8 μmols/gm) exchanges rapidly in the cold and presumably represents extracellular K. A previous estimate of extracellular K, based on equations (1) and (2), was 6 μmols/gm (6).

Fig. 2 illustrates the effects on the K^{42} efflux rate coefficient that were produced by the solutions used in a previous study of the Na fluxes (7). A K-free solution had little effect on the efflux rate coefficient, but a solution that contained 5 mM NaN_3, a Ca-free solution, or a solution that contained 8.5 mM K caused a prompt and reversible increase in the efflux rate coefficient. It is not known whether the increase in the K^{42} efflux rate coefficient that was produced by NaN_3 or by the Ca-free solution, was due to an increase in the K permeability of the fibers, to a depolarization of the fibers, or to a combination of these factors. It also is possible that some of the increase in the K efflux that occurred in the Ca-free solution was caused by spontaneous activity in

TABLE I

K CONTENTS OF DESHEATHED FROG SCIATIC NERVES SOAKED FOR VARIOUS PERIODS OF TIME IN RINGER'S SOLUTION AT 20°C OR AT 2°C

Nerves extracted in HNO_3.

Time in radioactive solution, *hrs.*	1–8	15	24
K content (μmols/gm dry) ± SD	196±16	177±14	165±11
No. of nerves	58	15	17

the nerve fibers. The effects of the solution with 8.5 mM K were probably due to depolarization of the membrane (14).

Fig. 3 shows the dependence of the initial rate of K^{42} uptake on the concentration of K in the bathing solution. These points were obtained with paired nerves; one member of each pair was soaked in standard Ringer's solution (2 mM K), and its mate was soaked in a solution that contained K at the concentration indicated on the abscissa. The nerves were soaked in their respective solutions for 2 hours before they were transferred to radioactive solutions for 2 hours. The nerves were extracted in HNO_3, and the intracellular K^{42} was estimated from equations (1) and (2). The data in Fig. 3 indicate that there are two components to the curve relating the rate of K^{42} uptake to the external K concentration, one being proportional to the external K concentration, the other showing saturation kinetics at low levels of extracellular K. If the linear portion of the curve is extrapolated to zero K concentration, the extrapolation passes close to the point at 2 mM K and intersects the ordinate at a level of 75 per cent, approximately. This indicates that when the external K concentration is 2 mM, the saturable component of the K influx accounts for about 75 per cent of the total K influx. The K influx into a nerve in Ringer's solution is about 20 μmols/(gm × hr.), which is equal to the Na efflux, approximately (7).

Fig. 4 summarizes the changes in the initial rates of K^{42} uptake that occurred when nerves were soaked in various solutions and then were allowed to recover in standard Ringer's solution. Paired nerves were used exclusively; the control nerve was soaked in oxygenated Ringer's solution, while the experimental nerve was soaked in one of the modified solutions. The symbols used in this figure refer to the conditions that prevailed during the treatment

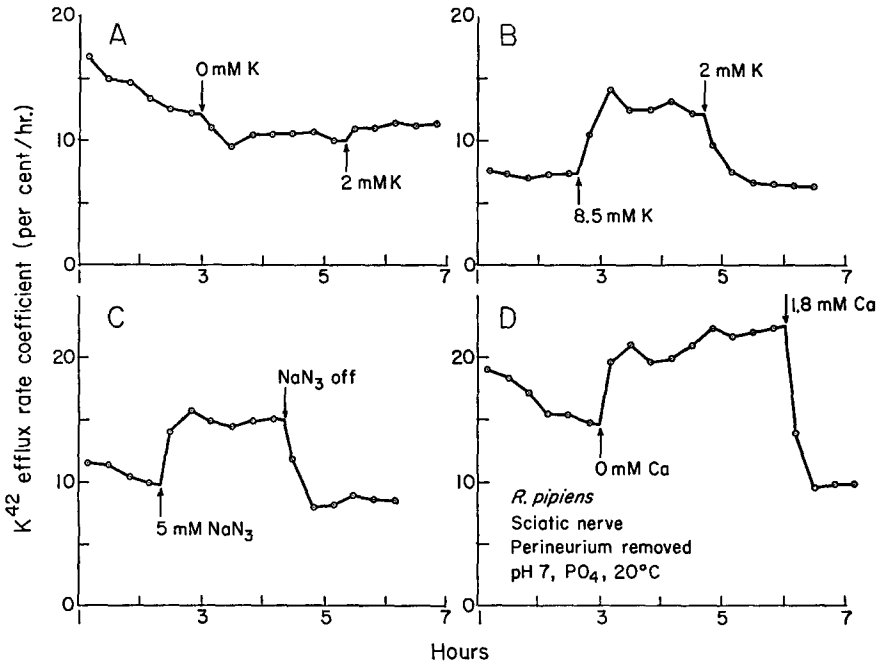

FIGURE 2. Effects of various solutions on the rate coefficient for the efflux of K^{42}. Ordinate, rate coefficient. Abscissa, time after beginning washout of K^{42}. All nerves were loaded with isotope by soaking about 4 hours at 20°C in Ringer's solution containing K^{42}. In each case, the initial washing solution was standard Ringer's fluid, and modified Ringer's fluid was substituted at the time indicated by the first arrow and removed at the second. A, Effect of K-free Ringer's solution. B, Effect of a solution containing 8.5 mM K. C, Effect of a solution containing 5 mM NaN_3. D, effect of a Ca-free solution.

period (0 to 5 hours). To obtain the recovery data (times greater than 5 hours), the nerves were first soaked for 5 hours in the appropriate experimental solution and then allowed to recover in standard Ringer's solution. The time courses of the changes in the initial rates of K^{42} uptake were followed in the manner described for Na^{22} (7), except that, in most cases, the nerves were exposed to K^{42} for 1 hour. The nerves were extracted in HNO_3, and the intracellular K^{42} was computed from equations (1) and (2). Anoxia and NaN_3 reversibly depressed the rate of K^{42} uptake, while Ca lack increased it about

20 per cent. After a nerve had soaked for a few hours in 5 mM NaN_3, the K influx was 4 to 5 μmols/(gm × hr.).

Ouabain or dinitrophenol inhibited the rate of K^{42} uptake about as strongly as did 5 mM NaN_3. Soaking nerves for 5 hours in a solution that contained

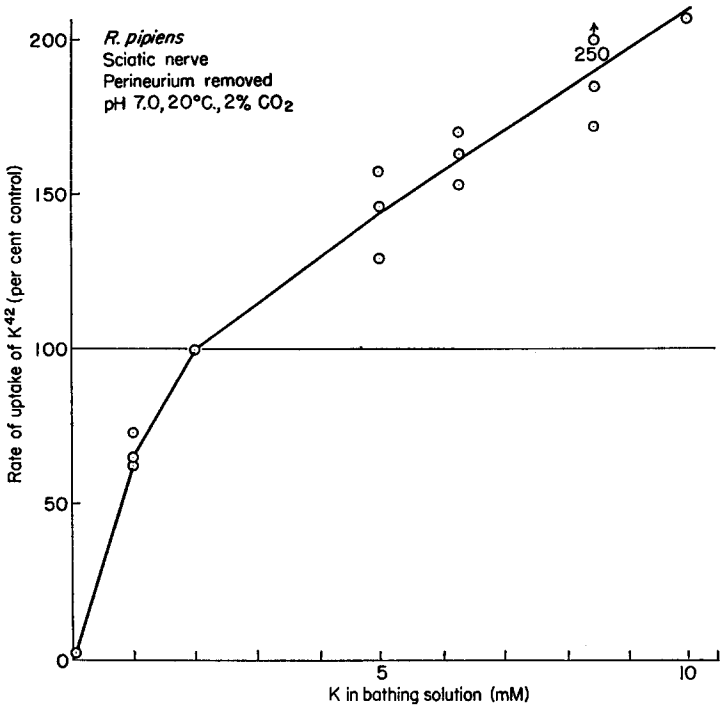

FIGURE 3. Initial rate of K^{42} uptake as a function of the K concentration of the bathing solution. Ordinate, initial rates of K^{42} uptake of nerves in the experimental solution expressed as a percentage of the initial rates of K^{42} uptake of control nerves in standard Ringer's solution (2 mM K). Abscissa, K concentration of the bathing solution. Each point represents one pair of nerves. The ruled line is a visual fit to the data. The average value of the initial rate of K^{42} uptake of the control nerves was 21.6 ± 3.6 μmols/(gm × hr.) (N = 19).

0.01 mM ouabain reduced their rates of K^{42} uptake from 15.8 ± 3.5 μmols/(gm × hr.) to 3.9 ± 0.4 μmols/(gm × hr.) (N = 6); soaking them for 5 hours in 0.2 mM α-dinitrophenol reduced their rates of K^{42} uptake from 20.1 ± 0.4 μmols/(gm × hr.) to 3.1 ± 0.4 μmols/(gm × hr.) (N = 4). During the 1st hour that nerves were exposed to 0.01 mM ouabain their rates of K^{42} uptake were reduced by about 50 per cent, and 0.2 mM α-dinitrophenol reduced the K influxes by about one-third during the first hour of exposure.

Approximately 75 per cent of the K influx, or 15 μmols/(gm × hr.), is due to active transport, since this fraction of the influx can be abolished by ouabain or by metabolic inhibition. This estimate of the size of the active

component of the K influx is consistent with the data in Fig. 3 which show that the saturable component of the K influx represents about 75 per cent of the total influx in Ringer's solution. The passive component of the K influx represents only 25 per cent of the total influx, or approximately 5 μmols/(gm × hr.).

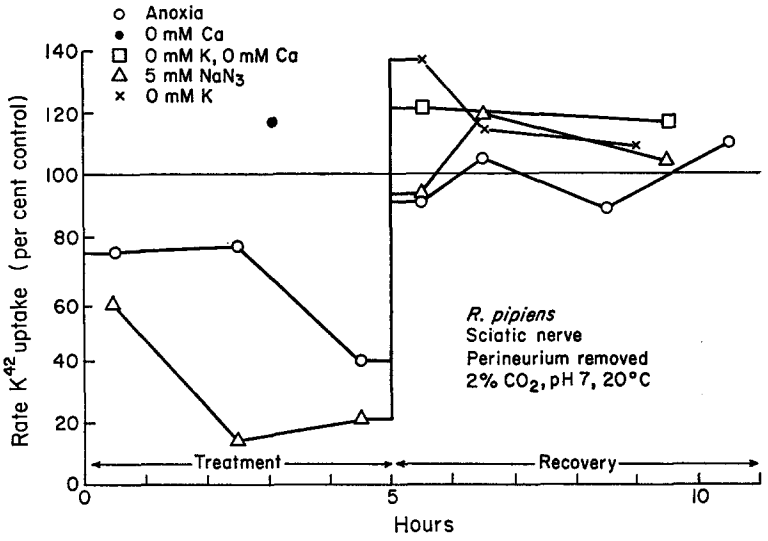

FIGURE 4. Time course of the changes in the initial rates of K^{42} uptake that occur when nerves are soaked in modified Ringer's solutions and then are allowed to recover in standard Ringer's solution. Ordinate, rates of K^{42} uptake of experimental nerves expressed as a percentage of the rates of K^{42} uptake of control nerves in oxygenated Ringer's fluid. Abscissa, time since beginning exposure to the experimental solution. The symbols indicate the solution used during the treatment period. The points are averages of from three to six determinations. The average value of the deviations of the individual data from the means was ± 10 per cent. In most cases, the nerves were exposed to the radioactive solution for 1 hour. However, to obtain the data in 0 mM Ca, to obtain the point, ×, plotted at 9 hours, and to obtain the point, △, plotted at 9.5 hours, the nerves were soaked for 2 hours in radioactive solutions. In each instance, the points are plotted at the midpoint of the period during which the nerves were soaked in the radioactive solution. The average value of the rate of K^{42} uptake by the control nerves was 21.8 ± 5.0 μmols/(gm × hr.) (N = 60).

When nerves are returned to Ringer's solution after they have been soaked for several hours in one of the experimental solutions shown in Fig. 4, their rates of K^{42} uptake are increased slightly above the control rates. The K^{42} efflux experiments indicate that the K^{42} efflux rate coefficient is normal during the recovery periods (Fig. 2). Since the K contents of the nerves are less than normal at these times, the K efflux also is less than normal. Therefore, the recover of the K contents of the nerves is due to a reduced K efflux as well

as to an increased K influx. It is useful to estimate the relative contribution of these two factors to the recovery of the K contents.

Consider first the recovery, in Ringer's solution, of a nerve that has been soaked for 5 hours in a solution containing 5 mM NaN_3. During the 1st hour of recovery the K influx is less than normal by 2 μmols/(gm × hr.); during the 2nd hour of recovery, the K influx is greater than normal by 4 μmols/(gm × hr.); during the 4th and 5th hours of recovery, the K influx is greater than normal by 0.6 μmols/(gm × hr.). If the K influx during the 3rd hour of recovery is assumed to be increased above normal by 3 μmols/gm × hr.), then in 5 hours the net K uptake due to the increased K influx is about 6 μmols/gm $(-2 + 4 + 3 + 2 \times 0.6)$. The K contents of nerves that have been soaked for 5 hours in 5 mM NaN_3 change from 83 ± 12 μmols/(gm(N = 36) to 134 ± 18 μmols/gm (N = 15) during 4 to 6 hours of recovery in Ringer's solution. Thus, the net K uptake that can be attributed to the increased K influx is only one-eighth of the total net K uptake.

Consider next the recovery of nerves that have been soaked for 2.5 hours in a K- and Ca-free solution. During the 1st hour of recovery the K influx is increased above normal by about 6 μmols/(gm × hr.); during the 5th hour of recovery, the K influx is increased above normal by 4 μmols/(gm × hr.). If the average increase in the K influx is assumed to be 5 μmols/(gm × hr.), then in 5 hours the net K uptake that is due to the increased K influx is about 25 μmols/gm. The K contents of nerves that have been soaked for 2.5 hours in a K- and Ca-free solution change from 67 ± 16 μmols/gm (N = 29) to 138 ± 7 μmols/gm (N = 7) during 5 hours of recovery in Ringer's solution. In this case, the net K uptake that is due to the increased K influx is about one-third of the total net K uptake.

When nerves are recovering from a soaking in a K-free solution, most of the net K uptake is due to the increase in the K influx. During the 1st hour of recovery the K influx is increased above normal by 10 μmols/(gm × hr.); during the 2nd hour the increase is 4 μmols/(gm × hr.); and during the 4th and 5th hours the increase is 2 μmols/(gm × hr.). If one assumes that during the 3rd hour of recovery the K influx is greater than normal by 3 μmols/(gm × hr.), then in 5 hours the net K uptake produced by the increased K influx is about 21 μmols/gm $[10 + 4 + 3 + 2 \times 2]$. This is 60 per cent of the measured net K uptake of 35 μmols/gm.

These results indicate that for small displacements of the ionic distribution, the reaccumulation of K may be due primarily to the enhanced K influx. For large displacements of the ionic distribution, however, the low K efflux is primarily responsible for the net uptake of K. It is also clear that the magnitudes of the increases in the K influxes are not proportional to the extents of the displacements of the ionic contents from the resting values, in contrast to the case with the Na effluxes (7).

It is important to determine whether, in recovering nerves, the active K influxes are directly related to the active Na effluxes. The active K influx in resting nerve was estimated to be 15 μmols/(gm $\times$ hr.). If it is assumed that the increases in the K influxes that occur during the recovery periods are due to increased rates of active transport, then the maximum active K influxes, which occur during the first few hours of the recovery period, can be estimated readily. When nerves are recovering after they have been soaked for 5 hours in a K-free solution or after they have been soaked for 5 hours in a solution that contains 5 mM NaN_3, the maximum active K influxes are about 25 μmols/(gm $\times$ hr.) and 19 μmols/(gm $\times$ hr.), respectively. When nerves are recovering after they have been soaked for 2.5 hours or for 5 hours in a K- and Ca-free solution, the maximum active K influxes are about 21 μmols/(gm $\times$ hr.) and 18 μmols/(gm $\times$ hr.), respectively. The Na effluxes under these conditions have been estimated to be 40, 60, 77, and 96 μmols/(gm $\times$ hr.), respectively (7). Approximately half of the Na efflux may be inhibited by 5 mM NaN_3 (7), so the active Na effluxes under these conditions are roughly 20, 30, 39, and 48 μmols/(gm $\times$ hr.), respectively.

There is no apparent correlation between these estimates of the active K influxes and of the active Na effluxes, and the results suggest that the active fluxes of these ions are not coupled in a stoichiometric manner. This indication of a lack of coupling between these fluxes is not particularly significant, since the active K influx could be accounted for by assuming that only part of the active Na efflux is coupled to it. If conditions could be found under which the active K influx exceeded the active Na efflux, the case for the absence of coupling between these active fluxes would be put on a firmer basis. To do this, experiments were carried out with Na-deficient solutions.

Previous results (7) have shown that in recovering nerves the time constant for the Na^{22} efflux is about 2 hours, which is equivalent to a half-time of 1.4 hours. If the K influx were tightly coupled to all of the Na efflux, then the K content of a nerve should recover with the half-time for Na^{22} exchange. If the K influx were not increased at all during the recovery time, then the K content of a nerve should recover with the half-time of 5 to 7 hours that is characteristic of K^{42} exchange. Following a 2.5 hour soaking in a K- and Ca-free solution, or a 5 hour soaking in a solution with 5 mM NaN_3, the K content of a nerve recovers with a half-time of 3 to 4 hours (6, 7). When nerves recover after having been soaked for 5 hours in a K-free solution, or after having been stimulated for 1 hour at 50 volleys/sec., the half-time for the recovery of the K contents is about 2 hours (2, 7), which is comparable to the half-time for Na^{22} exchange. This shows that when large changes in the ionic distribution of nerve have occurred, all of the Na efflux cannot be linked to inward K movement, and it shows that the recovery of the K contents is due to an increased influx and to a reduced efflux.

Na-Deficient Solutions Nerves were soaked for periods up to 10 hours in solutions in which part of the normal complement of NaCl had been replaced by isosmotic quantities of choline chloride, sucrose, or LiCl. At the

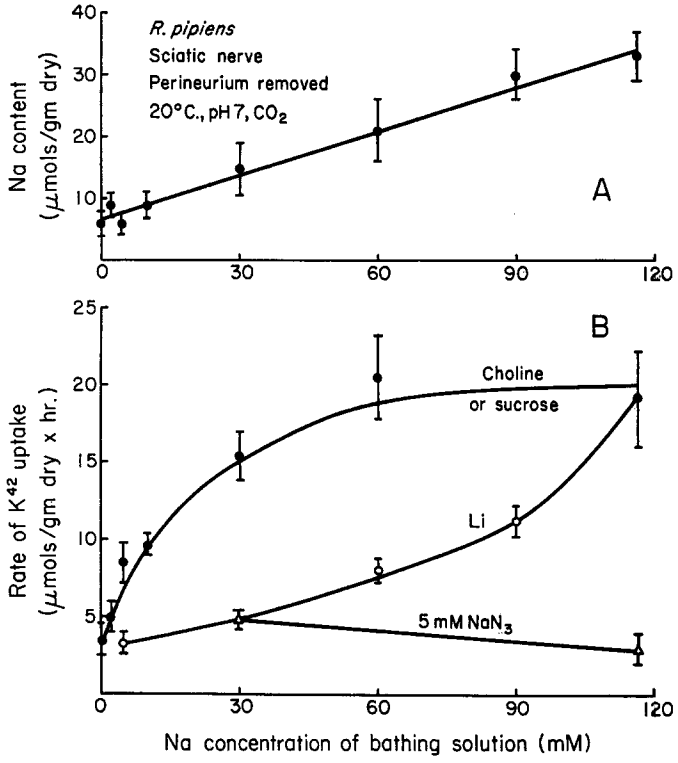

FIGURE 5. Steady-state values of the Na contents and initial rates of K^{42} uptake of nerves soaked in Na-deficient solutions. Ordinate, A, Na content, B, K^{42} taken up in 1 hour. Abscissa, concentration of Na in the bathing solution. Mean values ± SD. In A and the uppermost curve in B, the points at Na concentrations of 116 mM and 30 mM are based on twenty-five or more determinations; other points are averages of three to six measurements. The points at zero external Na were obtained using solutions buffered with tris. For the upper curve in B, choline chloride or sucrose was used as a substitute for NaCl; and for the middle curve, LiCl was used. Choline was used as the Na substitute to obtain the point in 5 mM NaN_3 at an external Na concentration of 30 mM. The curves are a visual fit to the data.

conclusion of each experiment, the nerves were washed for 1 hour at 2°C in solution W, and then were extracted for analysis. The Na contents of the nerves and the initial rates of K^{42} uptake fell to new steady-state values in a few hours. These steady-state values are presented in Fig. 5. When choline chloride or sucrose was substituted for 75 per cent of the NaCl in the bathing solution, the K contents of the nerves fell slightly: 10 ± 8 μmols/gm in 9

hours, (N = 13). When Li was substituted for Na in the bathing solution, the K contents of the nerves fell markedly (Table III). It is clear from Fig. 5 that the rate of K^{42} uptake is related to the Na concentration of the bathing solution. Shanes has observed a similar effect with toad nerves (12). The question to be decided is: Is the depression of the rate of K^{42} uptake due to a reduction of the Na efflux or is it due to another action of Na?

The Na content of a nerve varies linearly with the concentration of Na in the external solution (Fig. 5). The small amount of indiffusible Na indicated in Fig. 5 may be due to contamination of the nerve extracts with extraneous Na. The Na^{22} efflux rate coefficient is not affected by Na-deficient solutions. Therefore, in the steady state, the Na efflux, which is given by the product of the Na^{22} efflux rate coefficient and the Na content of a nerve, should be linearly related to the Na concentration of the bathing solution. The Na fluxes in a nerve in Ringer's solution are about 20 μmols/(gm × hr.) (7), and the normal K fluxes are also about 20 μmols/(gm × hr.) (Figs. 3 to 5). When a nerve is soaked in a solution in which 75 per cent of the NaCl has been replaced by sucrose or choline chloride, the Na fluxes should fall to about 5 μmols/(gm × hr.). Under these conditions, the K influx is 15 μmols/(gm × hr.), which is considerably higher than the Na efflux.

The active component of the K influx is also higher than the NaN_3-sensitive component of the Na efflux. Five millimolar NaN_3 inhibits about 50 per cent of the Na efflux from a nerve that is soaking in a solution containing 30 mM Na. The NaN_3-sensitive component of the Na efflux is, therefore, 2 to 3 μmols/(gm × hr.). Under these conditions, 5 mM NaN_3 reduces the K influx by about 9μmols/(gm × hr.) (Fig. 5); 0.2 mM dinitrophenol reduces the K influx by about 8 μmols/(gm × hr.) (Table II); and 0.01 mM ouabain reduces the K influx by about 6 μmols/(gm × hr.) (Table II). These three estimates of the size of the active K influx are two to three times greater than the estimate of the size of the NaN_3-sensitive component of the Na efflux. Therefore, it is unlikely that the effects of Na-deficient solutions on the active K influx are directly related to the decline in the Na efflux that occurs in such solutions.

Thus, under some conditions, the active Na effluxes in frog nerve exceed the active K influxes, and under other conditions the active K influxes exceed the active Na effluxes. In the steady state, the relation between the active Na efflux and the external Na concentration is linear, but the relation between the active K influx and the external Na concentration is hyperbolic. These results show that the active K influx is not stoichiometrically related to the active Na efflux.

The evidence for a lack of coupling between the Na efflux and the K influx would be strengthened if one could demonstrate an accumulation of K in exchange for an ion other than Na. Experiments were done as follows. Paired

nerves were soaked for 1 to 2 hours in a solution that contained 1.8 mM Ca, 2 mM K, and 30 or 60 mM Na. Choline chloride, sucrose, or LiCl was used as the substitute for NaCl. Then, one nerve of each pair was transferred to a K- and Ca-free solution with the same Na concentration. After 2.5 hours, both nerves were washed for 1 hour at 2°C in solution W and then were dried, weighed, and extracted. The differences between the Na contents and the differences between the K contents of the paired nerves were taken as a measure of the effect of the K- and Ca-free solution on the ionic distribution. To measure the recovery from an exposure to a K- and Ca-free solution, paired

TABLE II

EFFECTS OF OUABAIN AND α-DINITROPHENOL ON THE IONIC DISTRIBUTION OF NERVES IN AN Na-DEFICIENT SOLUTION

Inhibitor	Concentration	Change in ionic content		Rate of K^{42} uptake		
		Na	K	C*	X‡	C − X
	mM	*μmols/gm dry*		*μmols/(gm dry × hr.)*		
Ouabain	0.01	+15	−22	11.3	5.1	6.2
		±3	±3	±2.1	±0.6	±1.7
α-DNP	0.2	+16	−36	11.6	3.2	8.4
		±6	±8	±1.2	±0.2	±1.3

Mean values ± SD. Averages of four to seven determinations.
The solutions contained 30 mM Na and choline was used as the Na substitute.
The nerves were soaked for 5 hours in their respective solutions and the rate of K^{42} uptake was measured over the last hour of the soaking period.
* Control nerves.
‡ Experimental nerves.

nerves were soaked in that solution for 2.5 hours. Then, one nerve was prepared for analysis, and its mate was placed for 5 additional hours in an Na-deficient solution that contained K and Ca at their standard concentrations. The differences between the ionic contents of these paired nerves were taken as a measure of the extent of the recovery of the ionic distribution. The results are summarized in Table III. The K- and Ca-free solutions produced a loss of K from the nerves that was greater than the gain of Na. When the nerves recovered in solutions that contained choline chloride or sucrose, K was reaccumulated, and the gain of K was balanced by the loss of Na. In Li solutions, an additional loss of K occurred during the recovery period; the K exchanged for Li.

Although the data in Table III indicate that in frog nerve the net movements of K are coupled to the net movements of Na, they do not prove that the basis of this coupling is a single transport process in which the influx of K is stoichiometrically related to the efflux of Na. The changes in ionic contents

produced by K- and Ca-free solutions that contain 30 mM Na are less than half as great as the changes produced by K- and Ca-free solutions that contain 120 mM Na (7). This difference between the effects of the two solutions can be explained readily on the assumption that in the Na-deficient solutions fewer extracellular cations are available for exchange with intracellular K (little additional choline was taken up by the nerves from K- and Ca-free solutions). In other words, K will not be lost from a nerve unless an extracellular cation can move into the nerve to maintain electroneutrality. Similarly, K will not be reaccumulated by a nerve unless an intracellular cation leaves to maintain electroneutrality. If Na were the only cation to have entered a nerve during an experimental treatment, then one would expect that, during the recovery period, the amount of K reaccumulated could not exceed the amount of Na lost. Consequently, one interpretation of the data in Table III is that, because of the requirement for electroneutrality, the reaccumulation of K cannot occur unless another intracellular cation is available for exchange. The data do not prove that this cation must be Na.

TABLE III

IONIC MOVEMENTS PRODUCED BY K- AND Ca-FREE, Na-DEFICIENT SOLUTIONS

External solution*	Ion	Control contents‡	Change§ produced by 2.5 hrs. 0 mM K, 0 mM Ca	Change§ produced by 5 hrs.' recovery
30 mM Na	K	157±9	−33±9	+15±5
Sucrose	Na	17±5	+14±3	−10±3
30 mM Na	K	151±14	−28±8	+12±9
	Na	13±4	+5±4	−9±4
Choline	Choline	28±3	+8±3	
60 mM Na	K	125±7	−29±11	−20±11
	Na	19±3	+2±4	−2±7
Li	Li	57±5	+20±6	+14±5

Mean values ± SD; units are μmols/gm dry.

* This column indicates the Na concentration of the bathing solution and the substance used as the Na substitute.

‡ Ionic contents of nerves soaked 3 to 5 hours in Na-deficient solutions that contained K and Ca at their standard concentrations. Averages of twelve to twenty-three determinations, except choline, which is an average of four determinations.

§ Differences between ionic contents of paired nerves. Averages of six to nine pairs, except choline, which is an average of three pairs.

All nerves were washed for 1 hour at 2°C in solution W before being extracted for analysis. Further explanation in text.

Shanes and Berman (15) soaked toad nerves in isotonic NaCl for 16 hours and then allowed the nerves to recover in Ringer's solution for 24 hours. They reported that

K was taken up by the nerves during the recovery period but that no Na was lost. The experiments of Shanes and Berman were much longer in duration than those reported here. It has been my experience that for experiments lasting 10 hours or less, the total cation content of frog nerve remains nearly constant.

Lithium enters a nerve readily, but K is not reaccumulated when the recovery solution contains Li (Table III). This observation seems to be at odds

TABLE IV
UPTAKE OF K IN EXCHANGE FOR Li

Conditions				Ionic content*			Radioisotope uptake‡, 0.5 hr.			
Soak		Recovery					K^{42}		Na^{22}	
Solution	Time	Solution	Time	K	Na	Li	Recovery	Control§	Recovery	Control§
	hrs.		*hrs.*	*μmols/gm dry*			*μmols/gm dry*		*μmols/gm dry*	
0 mM K + 0 mM Ca + 60 mM Na + 60 mM Li	2.5	2 mM K + 1.8 mM Ca + 60 mM Na + 56 mM chol.	0	96±9	21±3	77±3				
			1	108±8	22±2	54±8	11.5±1.9	10.3±1.4	12.0±0.5	11.1±1.3
			5	140±4	23±2	22±6				
5 mM NaN_3 + 55 mM Na + 56 mM Li	5	Ringer's	0	86±11	39±4	76±5				
			1	79±6	42±4	43±4	9.4±2.4	12.0±1.9	25.3±3.1	20.8±1.5
			5	130±13	49±3	22±4				

Mean values ± SD.
* Average of four to twelve measurements.
‡ The uptake of radioisotope was determined during the 2nd half-hour of recovery (0.5 to 1 hr.) Average of four determinations using paired nerves.
§ Control nerves were kept in the recovery solution for the entire experiment.
All nerves were washed 1 hour at 2°C in solution W before being extracted for analysis. Further explanation in text.

with the previous conclusion. However, Fig. 5 shows that Li depresses the K influx; therefore, there is no reason to expect K accumulation in the presence of Li. An uptake of K in exchange for Li can be demonstrated if Li is removed from the recovery solution. This is illustrated in Table IV. To obtain the data in the upper part of the table, nerves were soaked for 2.5 hours in a K- and Ca-free solution that contained 60 mM Li and 60 mM Na. These nerves were then transferred to a recovery solution that contained K, Ca, 60 mM Na, and that employed choline, instead of Li, as the Na substitute. The recovering nerves accumulated K in exchange for Li, and their Na contents remained virtually unchanged throughout the experiment.

Keynes and Swan observed a similar phenomenon with frog muscle (10). These workers suggested that extracellular Na exchanged passively for intracellular Li, and that the Na then was extruded in exchange for extracellular K. A similar explanation of the data in Table IV cannot be ruled out entirely. However, the data in Table IV indicate that in the recovering nerves the active K influxes exceeded the active Na effluxes, and it seems unlikely that all of the K uptake was coupled to Na extrusion. The data on the right side of Table IV show the amounts of Na^{22} and K^{42} that were taken up by the nerves during the second half-hour interval of the recovery period. This interval was chosen, rather than the first half-hour, to enable Li, which inhibits K^{42} uptake, to be washed from the extracellular spaces. The uptakes of Na^{22} and K^{42} were about 12 μmols/gm. These nerves had been washed for 1 hour at 2°C in solution W. In nerves equilibrated with standard Ringer's solution and then washed for 1 hour in solution W, the intracellular Na^{22} contents are overestimated by 10 μmols/gm (7). In the present instance, the nerves were soaked in solutions that contained half the normal complement of Na, and the intracellular Na^{22} contents should have been overestimated by 5 μmols/gm. Therefore, the amount of Na^{22} that entered the fibers during the test period was 7 μmols/gm, which corresponds to an Na influx of 14 μmols/(gm × hr.). Since the Na contents of the nerves were in a steady state (Table IV, top), the Na influx should have been equal to the Na efflux. The Na^{22} efflux rate coefficient is about 60 per cent/hr. (7), and the Na efflux was, therefore, 10 μmols/(gm × hr.) [(22 − 5) × 0.6], which agrees with the estimate for the Na influx.

The K influx in these nerves was about 23 μmols/(gm × hr.). Approximately 75 per cent of this influx, or 18 μmols/(gm × hr.), should have been active K influx. If the active Na efflux was 50 per cent of the total Na efflux, or about 5 to 7 μmols/(gm × hr.), then the active K influx was clearly the larger of the two. On the other hand, if all the Na efflux were due to active transport, then the discrepancy between the sizes of the active K influx and the active Na efflux may not have been significant.

Nerves recovering from metabolic inhibition also could accumulate K in exchange for Li (Table IV, bottom). When nerves had been soaked for several hours in a solution in which half of the NaCl had been replaced by LiCl, the Na contents of the nerves were about 20 μmols/gm (Fig. 5). When the soaking solution also contained 5 mM NaN_3, the Na contents of the nerves rose to 39 μmols/gm in 5 hours (Table IV, bottom), a value close to the value of the Na contents of nerves in Ringer's solution. Therefore, to demonstrate that such nerves could accumulate K in exchange for Li, although net Na movements were small, the nerves were allowed to recover in standard Ringer's solution. The data in Table IV indicate clearly that the nerves accumulated K in exchange for Li. The Na contents of the recovering nerves

also increased by about 10 μmols/gm. Only half of this increase represented a change in intracellular Na, for the Na contents of the extracellular spaces should have increased by 5 μmols/gm when the Na concentration of the bathing solution was changed from 60 mM to 116 mM (7). The explanation suggested by Keynes and Swan for the reaccumulation of K by these nerves cannot be excluded because the active Na effluxes and the active K influxes were equal, approximately.

One explanation of the data in Table IV is that Li is substituting for Na in the ionic transport system, but the data in Table V argue against such an interpretation. Nerves were soaked for 4 hours in a solution in which all of

TABLE V

EFFECT OF Na ON K^{42} UPTAKE BY Li-LOADED NERVES

Concentration of recovery solution			Rate of K^{42} uptake	Li content
Na	Li	Choline		
mM	*mM*	*mM*	*μmols/gm dry × hr*	*μmols/gm dry*
0	0	116	2.9±0.5	57±4
0	30	86	3.3±0.7	90±4
30	30	56	10.1±3.5	74±8
30	0	86	15.4±4.6	46±6
90	30	0	11.9±0.9	70±3

Tris buffer. Each solution contained 1.8 mM $CaCl_2$ and 2 mM KCl.
Each number is an average (±SD) of the results of three measurements using nerves from different frogs. All nerves were washed 1 hour at 2°C in solution W before being extracted for analysis. Further explanation in text.

the Na had been replaced by Li. They were then transferred for 1 hour to one of the solutions indicated in the first column of Table V, and then the initial rate of K^{42} uptake was measured in this solution. It is clear that the uptake of K^{42} required Na in the external solution, and that Li could not substitute for Na in this regard. If the data in the fourth row of Table V are compared with the data in Fig. 5, one sees that Li in the nerve does not reduce the K^{42} uptake. The inhibitory action of Li appears to be due to its presence in the bathing solution.

When a nerve is soaking in a solution in which 75 per cent of the NaCl has been replaced by sucrose or choline chloride, the K influx exceeds the Na efflux. Since the K content of a nerve changes little under these conditions, it seems likely that K in the bathing solution is exchanging directly for K within the fibers. Support for this interpretation is furnished by the experiments shown in Fig. 6. Fig. 6A shows that the K^{42} efflux rate coefficient is not changed greatly when 75 per cent of the NaCl in Ringer's solution is replaced by an isosmotic quantity of sucrose. Similar results are obtained if the Na is

replaced by choline. This indicates that the K efflux is not coupled to the Na influx.

The experiment illustrated in Fig. 6B was more complex. The uppermost curve in Fig. 6B is that shown in Fig. 6A, and it was placed here to provide a baseline against which the other curves could be compared. The two lower curves in Fig. 6B were obtained from paired nerves. The left nerve was in Ringer's solution, and the right nerve was washed in an isotonic solution that contained 30 mM Na, sucrose, and the standard concentrations of KCl and

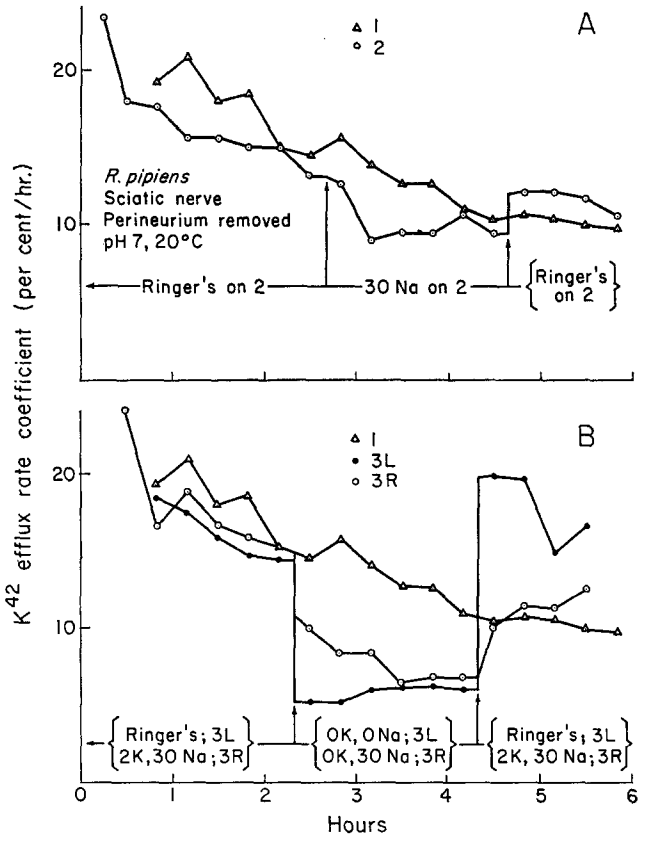

FIGURE 6. Effects of Na-deficient solutions on the K^{42} efflux rate coefficient. Ordinate, rate coefficient. Abscissa, time after beginning washout of K^{42}. Nerves loaded 4 hours in Ringer's solution containing K^{42}. A, nerves from different frogs, one washed with standard Ringer's fluid throughout the experiment. The second nerve was washed with Ringer's fluid initially, and at the time indicated by the first arrow, the washing solution was changed to one containing 30 mM Na. Ringer's solution was restored to the second nerve at the second vertical arrow. B, Effect of removal of external K on the K^{42} efflux rate coefficient of nerve in an Na-deficient solution. Tris buffer on 3L, 3R. Further explanation in text.

$CaCl_2$. At a time indicated by the first vertical arrow, the KCl was removed from the Na-deficient solution that bathed the right nerve, and an isotonic solution that contained only sucrose and $CaCl_2$ was placed on the left nerve. The original solutions were replaced at the time indicated by the second vertical arrow. When the KCl was removed from the Na-deficient solution that bathed the right nerve, the K^{42} efflux rate coefficient fell about 40 per cent in an hour. Measurements of the membrane potential were not made, but this decline in the K^{42} efflux rate coefficient may have been accompanied by an increase in the membrane potential.

When a nerve is soaking in standard Ringer's solution, removal of the K from the solution has little effect on the K^{42} efflux rate coefficient (Fig. 2), and the membrane potential increases only a few millivolts (9). Presumably, the small effect of a K-free solution on the K^{42} efflux rate coefficient, and on the membrane potential, is due to the fact that when a nerve is put into a K-free Ringer's solution the Na influx increases (7). When K is removed from an Na-deficient solution in which a nerve is bathing, the Na influx increases also, but the increase in the Na influx is much less than that observed when the K is removed from Ringer's solution.

To measure this, four pairs of nerves were used; the experimental member of each pair was soaked for 4 hours in a K-free solution that contained 30 mM Na and choline chloride, while the control nerve was soaked in a similar solution that contained 2 mM K. Then the rates of Na^{22} uptake were measured. The amount of Na^{22} taken up in 0.5 hour by the experimental nerves exceeded the amount taken up by the control nerves by 6.4 ± 0.4 μmols/gm.

When this experiment is carried out with standard Ringer's solution and K-free Ringer's solution, the increase in the amount of Na^{22} taken up in 0.5 hour by the experimental nerves is 16.9 ± 4.9 μmols/gm ($N = 6$) (7). This is two to three times larger than the increase that occurs in the Na-deficient solution. This suggests that the reason the K^{42} efflux rate coefficient declines when K is removed from an Na-deficient solution is that the Na influx does not increase sufficiently to enable the nerve to gain Na at a rate equal to the original K efflux. Therefore, the K efflux must decline to maintain the electroneutrality of the axoplasm (assuming that anions do not move). The nerve fibers should hyperpolarize if, as seems to be the case (14), the membrane potential is a major part of the driving force for K ions in the membrane.

Eventually, the rate coefficient of the right nerve (Fig. 6B), which was bathed in a K-free solution that contained 30 mM Na, attained a value only slightly higher than the rate coefficient of the left nerve, which was in a K- and Na-free solution. This indicates that the residual K efflux from the right nerve was not exchanging for extracellular Na and may have been accompanied by an intracellular anion.

DISCUSSION

Ussing (19) has derived a relation between the influx and the efflux of a monovalent cation that crosses a cell membrane solely under the influence of its own concentration gradient and the electric field. According to this relation,

$$M_{iP}/M_{oP} = (a_o/a_i) \exp (FV/RT) \quad (3)$$

where: M_{ip} = the passive influx of the ion
M_{op} = the passive efflux of the ion
a_o = the activity of the ion in the external solution
a_i = the activity of the ion in the axoplasm
V = the membrane potential in volts and R, T, and F have their conventional meanings.

It is useful to apply this relation to the Na and the K fluxes in resting frog nerve at 20°C to estimate the magnitude of the passive component of the K influx and the passive component of the Na efflux. The intracellular Na and K contents of frog nerves are about 40 μmols/gm and 160 μmols/gm, respectively. The intracellular water content of frog and toad nerves has been estimated to be 1.1 to 1.5 gm H_2O/gm (6, 15). The mean value of 1.3 gm/gm (dry) will be used for calculation. The intracellular Na and K concentrations, therefore, are 31 mM and 123 mM, respectively. When the external K concentration is 2 mM, the resting membrane potential of frog fibers is approximately 71 mv (8). If the activities of the ions are replaced by the concentrations, and if the Na influx and the K efflux of 20 μmols/(gm × hr.) are assumed to be passive, then the values calculated for the passive Na efflux and for the passive K influx are 0.3 μmol/(gm × hr.) and 5.3 μmols/(gm × hr.), respectively. This computed value for the passive K influx agrees with the K influx measured in nerves soaked for several hours in a solution that contained 5 mM NaN_3, 0.2 mM α-dinitrophenol, or 0.01 mM ouabain, and with the size of the linear component of the K influxes in Fig. 3. Thus, four independent estimates indicate that for a nerve in Ringer's solution about 75 per cent of the K influx, or 15 μmols/(gm × hr.), is due to active transport. When a nerve is soaking in a solution that contains 30 mM Na, the K influx is 11 to 15 μmols/(gm × hr.). The passive K influx still is about 5 μmols/(gm × hr.), and the active component is 6 to 10 μmols/(gm × hr.). Shanes (13), working with toad nerves, has also obtained good agreement between the computed value of the passive K influx and the K influx observed in asphyxiated nerves poisoned with iodoacetate.

In contrast to these rather consistent estimates of the size of the active K influx, the various ways of estimating the active Na efflux yield different results. The Ussing criterion indicates that practically all of the Na efflux is due

to active transport, but only about 50 per cent of the Na efflux is abolished by NaN_3, or dinitrophenol, or low temperatures, and only about 25 per cent is abolished by ouabain (7). For a nerve in Ringer's solution, the sizes of the active Na efflux defined by Ussing's relation, sensitivity to metabolic inhibition, or sensitivity to ouabain are 20, 10, and 5 μmols/(gm $\times$ hr.), respectively. For a nerve in a solution with 30 mM Na, the three estimates of the active Na efflux are 5, 3, and 1 μmols/(gm $\times$ hr.), respectively. It seems that more than one mechanism must contribute to the Na efflux. In nerves in Na-deficient solutions, the NaN_3-sensitive component of the K influx is two or three times greater than the NaN_3-sensitive component of the Na efflux. Therefore, it seems unlikely that this component of these fluxes is mediated by a single mechanism that transports Na and K in stoichiometrically related amounts. One conclusion that can be drawn from these results is that the active transports of Na and K are brought about by independent mechanisms.

The conclusion that the active K influx exceeds the active Na efflux in a nerve soaking in an Na-deficient solution is only as reliable as the estimates of the ionic fluxes. The validation of the estimates of the Na fluxes was presented in a previous paper (7). The estimates of the K influx, efflux, and net flux also form a self-consistent set of data. The rate coefficient for K^{42} efflux is 10 to 15 per cent/hr., the exchangeable K is about 160 μmols/gm, and the efflux is, therefore, 16 to 24 μmols/(gm $\times$ hr.), which agrees with the influx estimate of some 20 μmols/(gm $\times$ hr.). Since K-free solutions have little effect on the K efflux from a nerve and abolish the K influx, one would expect nerves exposed to K-free solutions to lose K at an initial rate of approximately 20 μmols/(gm $\times$ hr.). The observed loss in an hour is 11 $\pm$ 5 μmols/gm (N = 7). Calcium-free solutions double the K^{42} efflux rate coefficient and have little effect on the K influx. Therefore, a nerve should lose about 20 μmols K/gm during the 1st hour of soaking in a Ca-free solution. The observed loss is 18 $\pm$ 4 μmols/gm (N = 2). Since Ca lack affects only the K efflux, while K lack affects only the K influx, one would expect their combined effects to be additive. Therefore, during the 1st hour of soaking in a K- and Ca-free solution, a nerve should lose 40 μmols K/gm; the observed loss is 26 $\pm$ 10 μmols/gm in 0.5 hour (N = 6). During the 1st hour that a nerve is exposed to 5 mM NaN_3, the K influx is reduced about 40 per cent and the K^{42} efflux rate coefficient is increased about 50 per cent. The initial rate of loss of K produced by this agent would be 20 $\times$ (1.5 $-$ 0.6) = 18 μmols/(gm $\times$ hr.). The observed loss is 22 $\pm$ 5 μmols/gm (N = 8) in 1 hour. Since the estimates of the K influx and the K efflux and the observed changes in the K contents of a nerve comprise a self-consistent set of data, it seems unlikely that the estimates of the K fluxes are in serious error.

For nerves undergoing recovery, it is useful to compare the uptake of K that occurs in exchange for Li, with the uptake of K that occurs in exchange

for Na. After nerves have been soaked for 2.5 hours in a K- and Ca-free solution that contains 120 mM Na, their K and Na contents are 67 ± 16 μmols/gm and 149 ± 12 μmols/gm (N = 29), respectively (7). If such nerves are transferred to standard Ringer's solution for 5 additional hours, their K and Na contents change to become 138 ± 7 μmols/gm and 86 ± 12 μmols/gm (N = 7), respectively. During the recovery, the K contents of the nerves increased about 71 μmols/gm; if the normal K content is taken to be 160 μmols/gm, then recovery is 76 per cent complete (71/[160 − 67]). When the K is accumulated in exchange for Li, the increase in the K content is about 44 μmols/gm and is about 69 per cent complete (Table IV, top).

After nerves have been soaked for 5 hours in Ringer's solution with 5 mM NaN_3, their K and Na contents are 83 ± 12 μmols/gm and 114 ± 12 μmols/gm (N = 36), respectively. If such nerves are allowed to recover for 4 to 6 hours in standard Ringer's solution without NaN_3, their K and Na contents change to become 134 ± 18 μmols/gm and 76 ± 14 μmols/gm (N = 15), respectively. These figures for the K contents are similar to those shown in Table IV (bottom). These comparisons show that K can be taken up in exchange for Li about as readily as in exchange for Na.

The Li efflux rate coefficient, k, was calculated from the data in Table IV with the formula:

$$k = (1/5) \ln [(Li_0 - 5)/Li_5]$$

where: L_{i0} = the Li contents of nerves that had not recovered in an Li-free solution

L_{i5} = the Li contents of nerves that had recovered for 5 hours in an Li-free solution.

Because of the Li in the extracellular spaces, the data in Table IV overestimate by 5 μmols/gm the intracellular Li contents of those nerves that had not recovered in an Li-free solution. Therefore, when k was calculated, this number was deducted from the figure for the Li contents of those nerves. The value of k is 22 per cent/hr., which is about one-third of the value of the Na^{22} efflux rate coefficient (60 per cent/hr.). Therefore, for a given cation content the Li efflux should be about one-third of the Na efflux. In spite of this quantitative difference between the effluxes of Li and Na, the intracellular Li is nearly as effective as intracellular Na in producing an accumulation of K. This is because the extra Na efflux is largely balanced by an increased Na influx (7), and does not contribute to net Na movements or produce a large increase in the K influx.

One interpretation of these considerations and of the data in Table V is that Na plays two roles in K transport: one role, in which Li is an effective substitute for Na, is for intracellular Na to exchange for extracellular K; the second role that Na plays is highly specific but appears not to involve

exchange with K. In a strictly operational sense, this interpretation is true. However, the ability of intracellular Li or intracellular Na to exchange for extracellular K does not arise because the active K influx is tightly coupled to the active efflux of Na or Li. This is indicated by the fact that when nerves with a nearly normal K content are soaking in an Li-free and Na-deficient solution, the active K influxes are larger than the active Na effluxes and K appears to exchange for itself. It would be incongruous to speak of intracellular K substituting for intracellular Na in the extrusion process.

In nerves that are accumulating K in exchange for Na or Li, the K influx is increased only slightly above normal (Fig. 4, Table IV). The principal reason that Na- or Li-loaded nerves can accumulate K is not that these ions enhance appreciably the active inward transport of K, but simply that these ions, having displaced the K that is normally present in the axoplasm, thereby lower the K efflux to levels less than normal. It is the lowering of the K efflux that is mainly responsible for the accumulation of K by a nerve that contains large quantities of Na or Li in its axoplasm. Perhaps any cation that could cross the nerve membrane, and that had no deleterious effects on the metabolism or structure of the nerve, could exchange for extracellular K and produce K accumulation. This is not to say that the inward transport of K is responsible for the outward transport of Na, for it was shown previously (7) that the efflux of Na is not affected by a K-free solution. Rather, it appears that the active transport of K into the fibers and the active transport of Na out of the fibers may be mediated by different processes.

Although the active K influx is not stoichiometrically related to the active Na efflux when these fluxes are examined over a wide range of intracellular Na contents, the small increases in the K influxes of recovering nerves could be explained by assuming that these increases were due to the activation of a coupled transport process that could pump ions at a maximum rate of 5 to 10 μmols/(gm $\times$ hr.) and that was saturated when the Na content of a nerve increased about 50 per cent. Such a pump would be the major determinant of the net ionic movements in frog nerve only when the ionic contents had been displaced a small extent from their resting values. Therefore, the present results do not exclude the possibility that small changes in the ionic distribution, such as those produced by a bout of activity (2), activate a saturable transport process of limited capacity in which the influx of K is stoichiometrically related to the efflux of Na. However, if there be such a transport process in frog fibers, it still appears that the Na efflux is not dependent on external K, since the Na^{22} efflux from nerves recovering from stimulation is not greatly reduced by a K-free solution (7).

If Na and K are transported by independent mechanisms, then it is possible that the mechanisms are not electroneutral, as they seem to be in cephalopod axons (5), and the active transport of Na, or K, or both ions may

play a role in determining the membrane potential of frog nerve fibers, when they are not conducting action potentials. In this regard, Connelly (3) has suggested that the prolonged hyperpolarization of the membrane that follows the tetanization of frog nerve fibers may be due to an electrogenic Na pump; Lorente de Nó has maintained for many years that metabolic events play a direct role in determining the membrane potential of frog nerve (11); and Stämpfli has suggested that the resting potential of single frog nerve fibers may not be a K diffusion potential (17).

The suggestion was made that when a nerve is soaking in a solution that contains 30 mM Na, the K fluxes represent a self-exchange. The membrane potentials (17, 18) and the K fluxes of nerves in Ringer's solution are similar to the membrane potentials and the K fluxes of nerves in Na-deficient solutions. Therefore, neither the driving force for the K ions nor the K permeability of the nerve membrane appears to be greatly affected by an Na-deficient solution. If the state of the membrane is the same in an Na-deficient solution as in Ringer's solution, it is not unreasonable to suppose that under normal conditions the K efflux (which appears to be due to the passive movement of K ions since it is dependent on the membrane potential (14)) and the K influx (which is mostly due to active transport) represent an ionic exchange.

The notion that the K fluxes represent an ionic exchange that is independent of Na exchange is supported by the effects of temperature on the ionic fluxes and on the ionic distribution. At 2°C the Na fluxes are one-half to one-third as large as at 20°C (7) and the K fluxes are one-fifth to one-sixth as large as at 20°C (Fig. 1). The K content of frog nerve (and presumably the Na content also) is well maintained at 2°C (Table I). This indicates that the K influx and the K efflux have the same temperature coefficient and that this temperature coefficient is different from that of the Na fluxes. This suggests that the Na and K movements are due to different processes and supports the interpretation that K is exchanging for itself across the nerve membrane.

If it is true that K exchange across the membranes of frog nerve fibers occurs independently of Na exchange, then one possible explanation for the increases in the Na influx that occur when nerves are soaked in K-free solutions or are exposed to metabolic inhibitors is as follows. One could assume that K movements through the membrane occurred along charged sites whose specificity for K was dependent on the cellular metabolism. In the absence of K, or during metabolic inhibition, any cation of appropriate size present in the external solution could move along the sites. This proposal is similar to Shanes's Na exclusion pump, except that there is no need to assume a hidden influx of Na and a rapid turnover of the pump. However, such a scheme does not explain why Na is required for K transport. This would re-

quire the additional assumption that Na is important to the membrane structure, or that Na is a kind of cofactor in K transport. The participation of an Na-dependent ATPase in K transport is one possibility (16). The transport of several non-electrolytes by the frog intestine (4) and the transport of amino acids by calf thymus nuclei (1) are Na-dependent, and in these cases it seems unlikely that the non-electrolytes are exchanging for the Na ion.

The conclusions drawn from these studies are: both Na and K are actively transported in frog nerve, but probably by different mechanisms; because of the low anion permeability of the nerve membrane and because of the requirement for electroneutrality, K accumulation cannot occur unless another intracellular cation, not necessarily Na, is available for exchange; and Na plays a specific, but unknown, role in K transport.

I wish to thank Miss Virginia Haws, Miss Florence Ling, and Mrs. Shelley Tobias for their patient help and capable technical assistance, and to thank Dr. Frank Brink, Jr., and Dr. C. M. Connelly for reading the first draft of the manuscript.
Received for publication, October 5, 1962.

LITERATURE CITED

1. ALLFREY, V. G., MEUDT, R., HOPKINS, J. W., and MIRSKY, A. E., 1961, Sodium dependent "transport" reactions in the cell nucleus and their role in protein and nucleic acid synthesis, *Proc. Nat. Acad. Sc.*, **47**, 907.
2. ASANO, T., and HURLBUT, W. P., 1958, Effects of potassium, sodium, and azide on the ionic movements that accompany activity in frog nerves, *J. Gen. Physiol.*, **41**, 1187.
3. CONNELLY, C. M., 1959, Recovery processes and metabolism of nerve, *Rev. Mod. Physics*, **31**, 475.
4. CŠAKY, T. Z., 1961, Significance of sodium ions in active intestinal transport of nonelectrolytes, *Am. J. Physiol.*, **201**, 999.
5. HODGKIN, A. L., 1959, Ionic movements and electrical activity in nerve and muscle, *Proc. Roy. Soc. London, Series B*, **148**, 1.
6. HURLBUT, W. P., 1958, Effects of azide and chloretone on the sodium and potassium contents and the respiration of frog sciatic nerves, *J. Gen. Physiol.*, **41**, 959.
7. HURLBUT, W. P., 1963, Sodium fluxes in desheathed frog sciatic nerves, *J. Gen. Physiol.*, 1963, **46**, 1191.
8. HUXLEY, A. L., and STÄMPFLI, R., 1951 *a*, Direct determination of membrane resting potential and action potential in single myelinated nerve fibers, *J. Physiol.*, **112**, 476
9. HUXLEY, A. L., and STÄMPFLI, R., 1951 *b*, Effect of potassium and sodium on resting and action potentials of single myelinated nerve fibers, *J. Physiol.*, **112**, 496.
10. KEYNES, R. D., and SWAN, R. C., 1959, The permeability of frog muscle fibers to lithium ions, *J. Physiol.*, **147**, 626.

11. LORENTE DE NÓ, R., 1947, A study of nerve physiology Part I, *Studies from the Rockefeller Institute for Medical Research*, **131**.
12. SHANES, A. M., 1956, Distinction between effects on metabolic transport and passive transport of ions, *Science*, **124**, 727.
13. SHANES, A. M., 1957, Ionic transport in a vertebrate nerve, *in* Metabolic Aspects of Transfer Across Cell Membranes, (Q. R. Murphy, editor), Madison, University of Wisconsin Press, 127.
14. SHANES, A. M., 1958, Electrochemical aspects of physiological and pharmacological action in excitable cells. I. The resting cell and its alteration by extrinsic factors, *Pharmacol. Rev.*, **10**, 59.
15. SHANES, A. M., and BERMAN, M. D., 1955, Penetration of the desheathed toad sciatic nerve by ions and molecules. I. Steady state and equilibrium distributions, *J. Cell. and Comp. Physiol.*, **45**, 177.
16. SKOU, J. C., 1961, The relationship of a (Mg^{2+} + Na^{+})-activated K^{+}-stimulated enzyme or enzyme system to the active, linked transport of Na^{+} and K^{+} across the cell membrane, *in* Membrane Transport and Metabolism, (A. Kleinzeller and A. Kotyk editors), New York, Academic Press Inc., 228.
17. STÄMPFLI, R., 1959, Is the resting potential of Ranvier nodes a potassium potential? *Ann. New York Acad. Sc.*, **81**, 265.
18. STRAUB, R., 1956, Die Wirkung von Veratridin and Ionen auf des Ruhepotential markhaltiger Nervenfasern des Frosches, *Helv. Physiol. and Pharm. Acta*, **14**, 1.
19. USSING, H. H., 1949, The distinction by means of tracers between active transport and diffusion, *Acta. Physiol. Scand.*, **19**, 43.