HOW DOES REDUCED EXTERNAL K^+ CONCENTRATION AFFECT THE RATE OF Na^+ EFFLUX? EVIDENCE AGAINST THE K-Na COUPLED PUMP BUT IN SUPPORT OF THE ASSOCIATION-INDUCTION HYPOTHESIS

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• *Recent frog-muscle studies produced the following findings: 1. Contrary to the theory of K^+-Na^+ coupled pump, reduction of external K^+ concentration to near zero did not significantly reduce the rate of efflux of the fraction of cell Na^+ conventionalty regarded as rate-limited by membrane permeability. 2. Reduction of external K^+ concentration profoundly reduced the rate of the efflux of this fraction only if the muscles were exposed to the low K^+ while being loaded with radioactive Na^+. 3. The data indicate that the fraction of Na^+ efflux which in normal cells at room temperature has a half-time exchange ($t_{½}$) of **20-40** min is not rate-limited by membrane permeability but by desorption from cellular adsorption sites. Surface-limited Na^+ exchange between free Na^+ in the cell and the external environment is represented by a faster fraction with a $t_{½}$ of 2 to 4 min. **4.** The data further indicate that the slow-down of the rate of efflux of the (slow) fraction arises from a cooperative shift of those β- and γ-carboxyl groups from adsorbing K^+ to adsorbing Na^+ when external K^+ concentration is reduced below a critical level. The enhanced adsorption energy of the newly adsorbed Na^+ raises the activation energy, hence a slower rate of exchange is seen as a slow-down in the "efflux curves." It is therefore only when free labeled Na^+ is present in the cell water and thus available to the newly emerging Na^+ adsorption sites that the effect of low external K^+ can be visualized in a labeled-Na^+ efflux study. Application of low K^+ Ringer's solution after free labeled Na^+ in and out of the cells has been washed away only causes enhanced adsorption of non-labeled Na^+, which is not detected in isotope efflux study.*

INTRODUCTION

According to the membrane theory, the bulk of intracellular K^+ and Na^+ ions exist in a free state. The steady levels of these ions are maintained by pumps in the cell membrane which actively transport K^+ ions into and Na^+ ions out of the cell in a coupled, cyclic manner.[1] This theory is based on the observation that the Na^+-ion efflux rate in a number of tissues is related to the K^+-ion concentration in the bathing solution. Thus a reduction of external K+-ion concentration has been reported to cause a slowing down of Na^+-ion efflux whereas an increase of K+-ion concentration hastens it.[2-6]

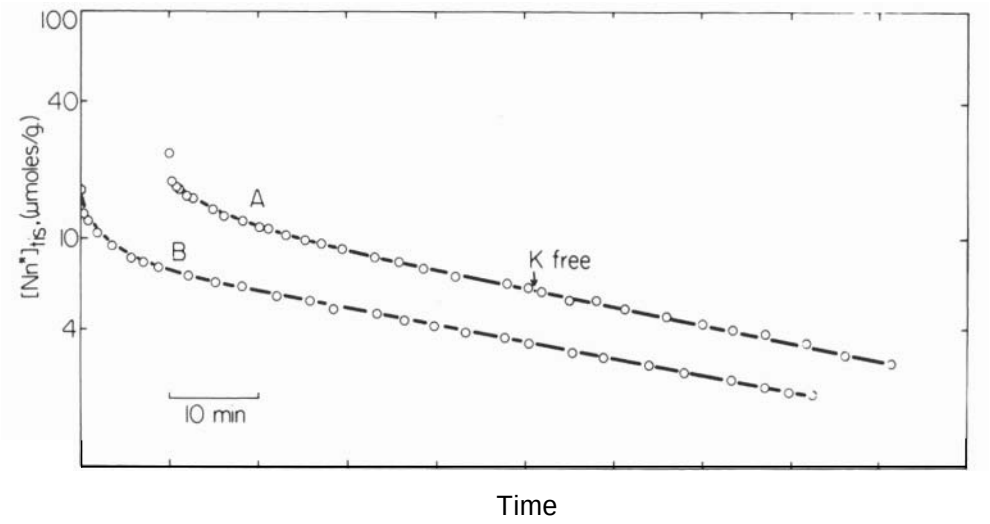

FIGURE 1. Lack of effect of lowering external K^+-ion concentration on the rate of Na^+-ion efflux after the loaded muscle had been washed in normal Ringer's for 40 min. Control and experimental muscles were pairs from the same frog incubated in the same ^{22}Na-tagged, Ringer's-GIB medium and otherwise treated in the same way. Wash-out solution was normal Ringer's solution (K = 2.5 mM) for both experimental (A) and control (B) muscle until the 40th min, after which the experimental muscle alone was switched to K-free washing solution.

According to the association-induction hypothesis, the intracellular ions (and other solutes) fall into two categories: interstitial and adsorbed. Interstitial ions are found in the cell water in the free state. This is not the case, of course, for ions adsorbed on protein and other macromolecules.[7–10] Evidence is gathering[10–12] that provides additional support for the earlier suggestion that the slower-exchanging fraction in the Na^+-ion efflux curve is rate-limited not by membrane permeability but by desorption.[7,10] In this view, the dependence of Na^+-ion efflux on external K+-concentration reflects the displacement of adsorbed Na^+-ion by the K+-ion and vice versa.[13-15]

This report will present results of experiments that may offer help in choosing between the two models.

MATERIALS AND METHODS

Intact sartorius muscles of leopard frogs (Rana *pipiens pipiens,* Schreber) were used in all experiments. All chemicals used were of cp grade. Carrier-free ^{22}Na was obtained from Nuclear Science and Engineering, Pittsburgh (batch S-2,23).

Muscles dissected sterilely were loaded with isotope by soaking at 25°C for a specified length of time in a sterile modified Ringer's-GIB medium mixture that permits long-term ***in vitro*** preservation at 25°C of frog muscle cells.[16] For studies on the effect of duration of incubation in ^{22}Na-containing K-free solution, we pre-

pared a single batch of stock solution and used 3 ml of this solution for each sartorius muscle (weighing less than 100 mg). The solutions were not reused. The solution used for washing was basically a modified Ringer's-phosphate solution, described elsewhere (see ref. 7, Appendix H).

The method of studying the efflux of labeled ions from those muscles has been described in full elsewhere.[7,17] With this method, in brief, isotope-loaded muscles are mounted in the bottom part of a narrow U-tube that fits into the "well" of a y-scintillation counter. A continuous stream of Ringer's solution flows through the U-tube while the remaining radioactivity in the tissue is being continuously monitored. In all cases, both loading and washout were carried out in a constant-temperature room at 25" ± 1°C.

RESULTS

Effect of Low External K+-ion Concentration on the Na+-ion Efflux of a Normal Muscle

Figure 1 shows that after 40 min of washing in normal Ringer's solution (2.5 mM K), a switch of the washing solution to a low K Ringer's (K+-ion concentra-

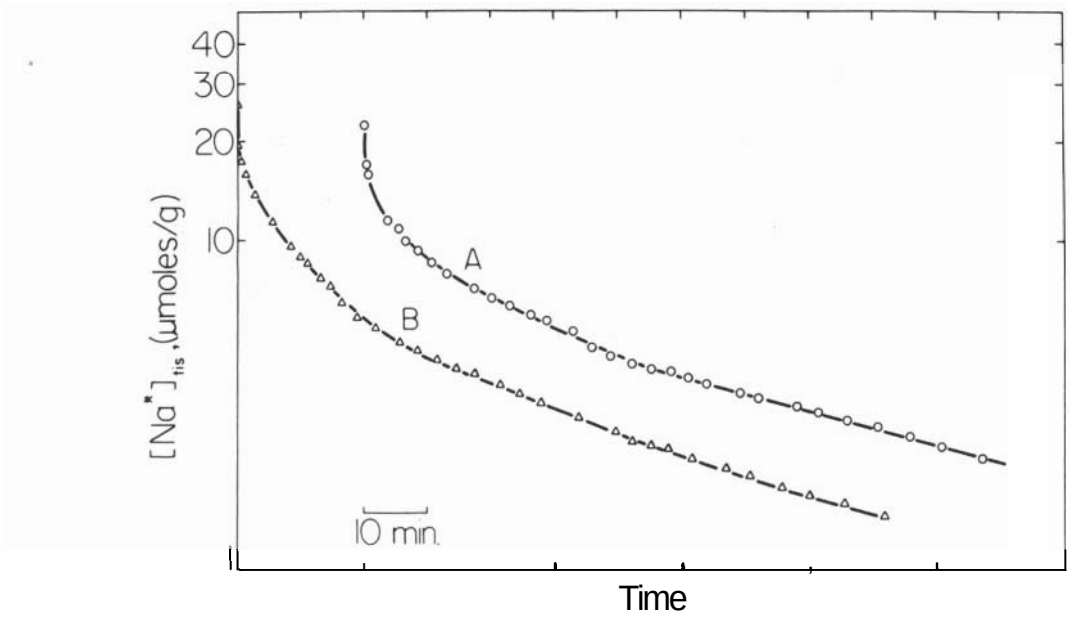

FIGURE 2. Effect of lowering K+-ion concentration in the washing solution on the Na+-ion efflux when the K-free washing solution was applied from start of the washing. Experimental (A) and control (B) muscle were paired and incubated in the same labeled normal Ringer's-GIB solution at 25°C for 24 h. Control muscle was washed in normal Ringer's solution (K^+, 2.5 mM) while experimental muscle was washed in K-free Ringer's solution from the start.

tion lower than 0.01 mM, henceforth referred to as K-free) produced no discernible change in the slope of the Na-efflux curve even after another 40 min of application of the K-Ringer's. This lack of effect is evident by comparison with the immediately preceding section of the same efflux curve or by comparison with the efflux of the control muscle. In other experiments I could detect no change in the efflux rate as long as 90 min after switching to a K-free washing solution. This indifference is at variance with the conclusion drawn by Keynes.[3]

In Fig. 2, a ^{22}Na-loaded sartorius muscle was, from the start, washed in K-free Ringer's, whereas its control pair was washed in normal Ringer's. No change was observed for the first 50 min, after which the efflux rate of the muscle washed in K-free solution showed a moderate slow-down. The half-time of exchange ($t_{1/2}$) of this portion of the curve is 60 min; the corresponding $t_{1/2}$ for the control muscle, 50 min.

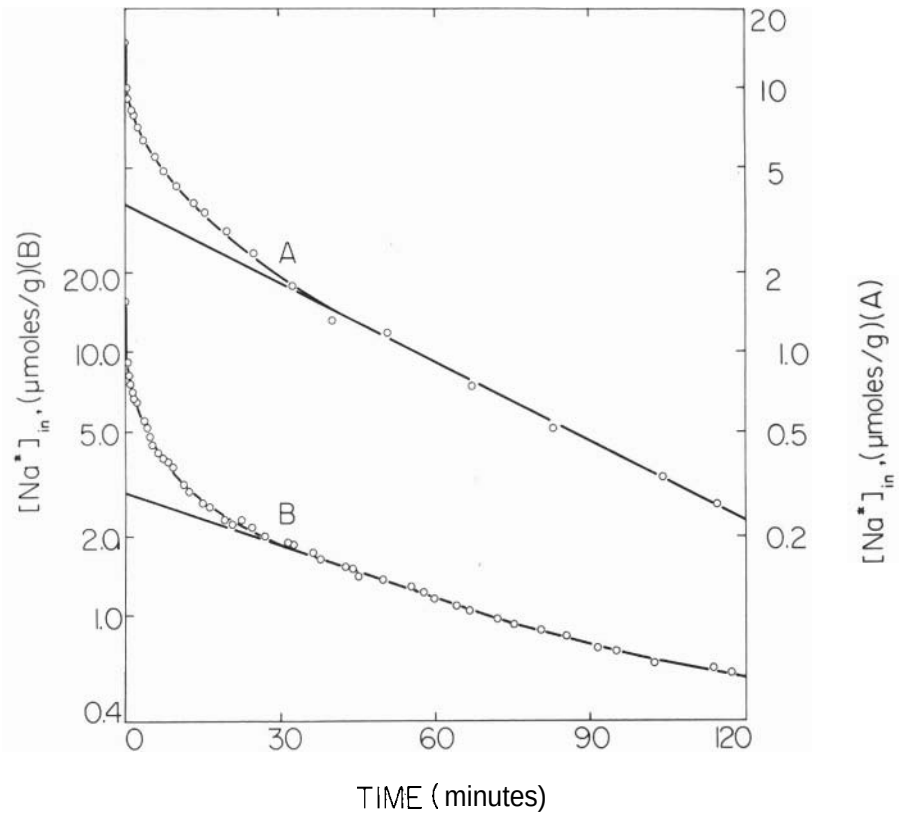

FIGURE 3. Effect of 10-min exposure to a ^{22}Na-containing, K-free solution on the subsequent efflux rate. The control muscle (A) was exposed for 10 min to a ^{22}Na-containing normal K-Ringer's solution; its pair, the experimental muscle (B), was also exposed for 10 min to a ^{22}Na-containing, K-free Ringer's solution.

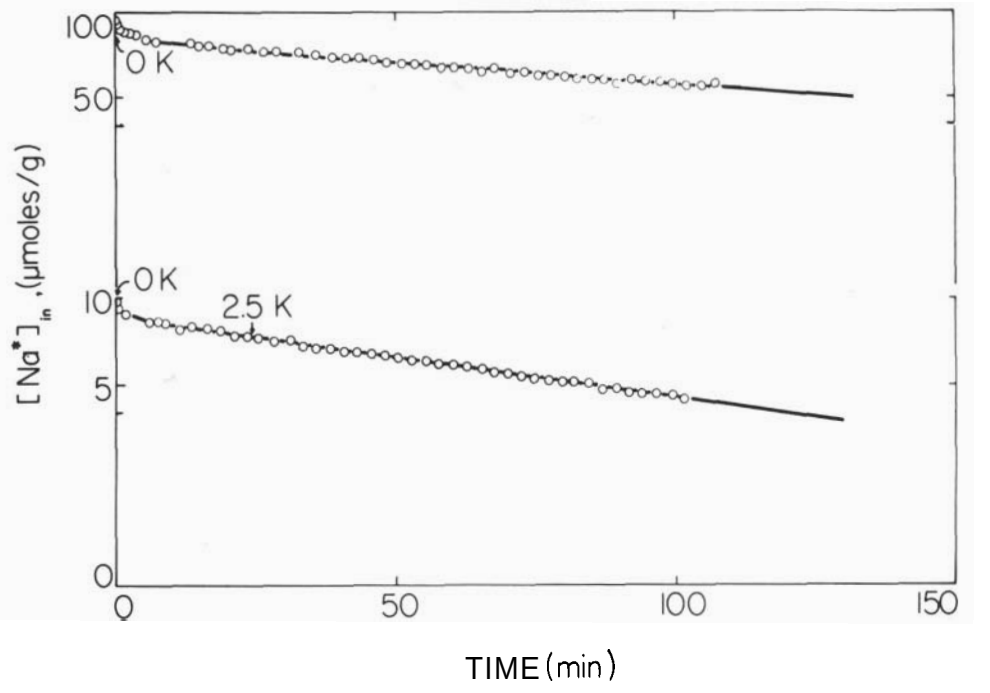

FIGURE 4. Effect of increasing the K^+-ion concentration from near zero to 2.5 mM on the Na^+-ion efflux after equilibration of the muscle in a K-free Ringer's solution. Control and experimental muscles were pairs. Both were incubated for 2 days in 250 ml of a K-free Ringer's-GIB medium at 25°C before transfer to a small flask containing 2 ml of ^{22}Na-labeled, K-free solution for 18 h (25°C). The control muscle was washed with K-free Ringer's solution entirely whereas the experimental muscle was first washed in K-free Ringer's solution for 23 min, at which time the washing solution for the experimental muscle was switched to a normal Ringer's solution ($K = 2.5$ mM).

Effect on the *Na^+-ion Efflux* Rate of Pre-incubation in a K-poor Ringer's Solution Containing *^{22}Na*

A low K+-ion concentration in the washing solution had little or no effect on the Na^+-ion efflux rate of muscles previously loaded in a ^{22}Na-labeled Ringer's containing a normal amount of K^+ ion. A lowering of K+-ion concentration in the loading solution, however, produced marked changes. Even a short exposure (10 min) to a ^{22}Na-containing K-free Ringer's produced significant slowing of the efflux during a subsequent washout with a low K Ringer's when compared to its control pair, which had been pre-incubated in a ^{22}Na-containing normal Ringer's solution and washed out in the same K-free Ringer's solution (Fig. 3).

With longer pre-incubation in a ^{22}Na-containing, K-free Ringer's, the slowing of the Na^+-ion efflux became more pronounced ($t_{1/2}$ = 190 min) (Fig. 4). In this case, a switch of washing solution from low K (0.01 mM) to normal K (2.5 mM), increased the efflux rate ($t_{1/2}$ = 110 min). But this increased rate was far short of

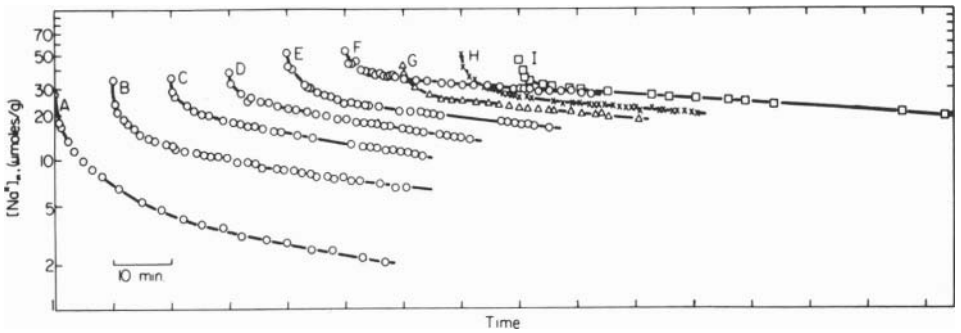

FIGURE 5. Effect of the duration of exposure to ^{22}Na-containing, K-free solution on the efflux rate. From left to right, the duration of incubation in the K-free, ^{22}Na-containing solution is seen to increase thus: A, 40 min; B, 2.5 h; C, 3.5 h; D, 4.5 h; E, 6 h 23 min; F, 8 h; G, 8 h 37 min; H, 12 h 42 min; and I, 18 h 40 min.

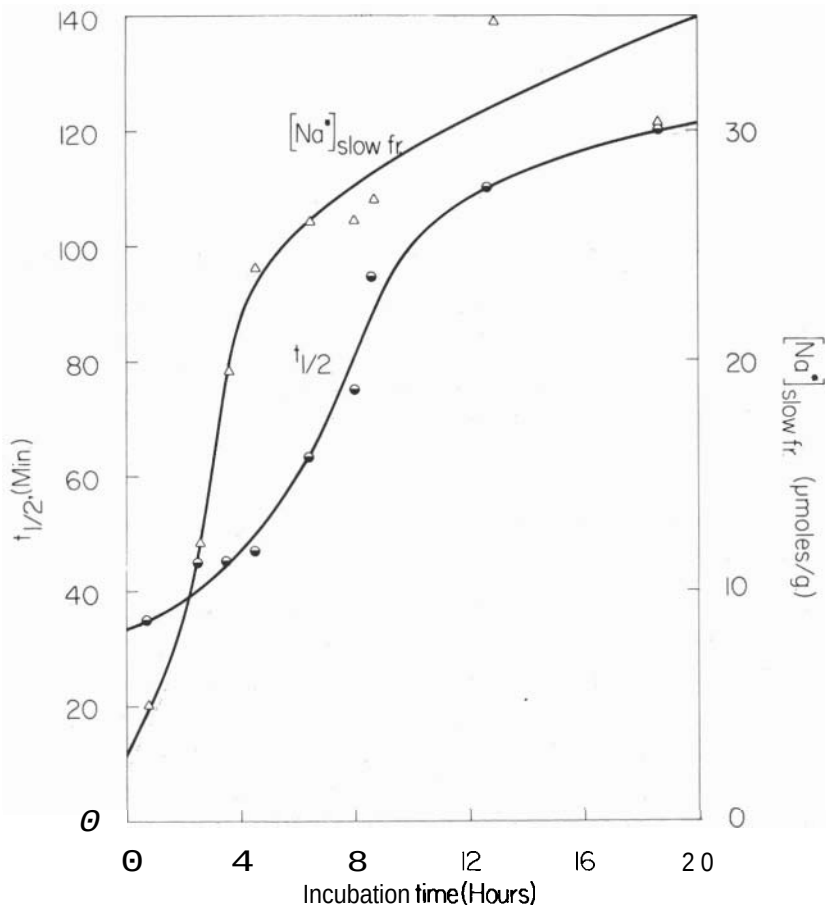

FIGURE 6. Relation of duration of incubation in a ^{22}Na-containing, K-free solution on magnitude of slow fraction, $Na^{+}_{slow\ fr}$, and apparent $t_{1/2}$ of efflux curve. Data are the same as in Fig. 5.

the efflux rate of a muscle loaded and washed in normal Ringer's solution, which usually has a $t_{½}$ of 20-40 min.

It follows that the Na^+-ion efflux rate in the low K-ion environment is primarily determined by what occurs in the isotope-loading process, and is indifferent to or only weakly influenced by the K+-ion concentration in the washing solution.

Effect on the *Na^+-ion Efflux* Rate of Duration of Pre-incubation in a *^{22}Na-containing*, Low *K* Ringer's

Sartorius muscles were pre-incubated in K-free ^{22}Na-labeled Ringer's solution at 25°C for 10 min to 3 days, and the efflux of each sample was followed. The data obtained are presented in Fig. 5. As the duration of pre-incubation increased, there was a progressive change in the slopes of the "main" portion of the curves despite use of the same K-free washing solution in all cases.

From the first point of each curve shown in Fig. 5, it is clear that with increasing pre-incubation there occurred a progressive increase of labeled Na^+-ion uptake. With this increase, there was a corresponding slowing down of the Na^+-ion efflux.* A plot of the ($t_{½}$) of the main portion of the curve of Fig. 5 against the duration of pre-incubation is shown in Fig. 6. It took more than 12 h in a 25°C K-free solution to complete the change in the effiux rate of the Na^+-ion. Figure 6 also presents a plot of the magnitude of the slow fraction of Na+ obtained by extrapolating to zero time against the duration of pre-incubation.

DISCUSSION

The Coupled K-Na Pump

Hodgkin and Keynes presented an enticing picture of how the coupled K-Na pump can act to restore K-depleted and Na-filled cells to normal after a burst of activity.[1] However, according to basic membrane theory, this pump is not supposed to function only during immediate cellular recovery from activity; rather, it is supposed to represent the fundamental mechanism in maintaining the ionic distributions in a resting cell. The function of the coupled pump under the latter condition becomes somewhat obscure.

To my knowledge, the proponents of the membrane theory at one time proposed that intracellular Na^+-ion is kept at low level by a pumping mechanism at

***It should be pointed out that there is no conflict between the present finding and the reported[18,19] *rise* of Na^+-ion efflux with the increase of total intracellular Na^+-ion content. What is being discussed here is the efflux rate constant and not the total efflux. The efflux rate constant, k, is related to $t_{½}$ by the relation, $k = \ln 2/t_{½}$, and is in units of sec^{-1}. Total efflux, on the other hand, is equal to $k[Na]_{in}$ and is given in units of moles, leaving a cm^2 cell surface per sec where $[Na]_{in}$ is the intracellular Na^+ ion concentration.**

the expense of metabolic energy.[20] Presumably this expenditure of energy is justified because a specific low Na^+-ion concentration is part of the internal milieu and essential for the continued well-being of the cell. The postulated Na pump could be seen in that light as one of the homeostatic mechanisms. But a rise of external K+-ion concentration beyond the normal range would tend to increase the intracellular K+-ion concentration and decrease the intracellular Na^+-ion concentration, thereby disturbing the homeostasis. Therefore if the function of the Na pump is to preserve homeostasis, such a decrease would call for a slow-down of the outward Na pump.

Nevertheless, according to the proposed K+-in, Na^+-out cyclic mechanism linking the operation of the Na pump to the external K+-ion concentration; a rise of external K+-ion concentration beyond the normal range is expected not to slow but to hasten the Na pump. By that logic it would seem that the pump is not acting to preserve a specific low intracellular Na^+-ion concentration. The pump, rather, at the expense of energy, is responding to the abnormally high external K^+ ion concentration by further lowering intracellular Na^+-ion concentration. This new postulation of coupled pump does not seem to agree with the original purpose in postulating the Na pump; namely, homeostasis.

Similarly, when the external K^+ ion concentration is lowered to a level below normal, the Na^+-ion concentration in the cell tends to rise. Once again, if the Na pump is to function to maintain homeostasis, this is the time when it should counteract the tendency by pumping harder. Again, however, the postulated coupling between Na pumping rate and external K^+ ion concentration would lead to just the opposite situation. It would reduce the pumping rate.

In short, while the model of the coupled K-Na pump may be plausible in explaining restoration of cells during or closely following activity, it is difficult to understand as the cell mechanism maintaining ion distribution in resting cells. The data given in Figs. 2 and 3 show that even as a mere rephrasing of experimental findings, the model in question has only limited factual support.

The Association-Induction Hypothesis

According to the association-induction hypothesis, interstitial solute found in the cell water is, as a rule, at a lower concentration than that in the external medium. This is so because of cell water existing in a state of polarized multilayers (see ref. 7, chapters 2, 12; also refs. 9, 10, 21) is characterized by a lower entropy (and/or enthalpy) of solutes like Na^+. A second type of solute is adsorbed on sites carried by macromolecules inside the cells. The predominant sites are those provided by the cell proteins.[22,23]

Na+ and K^+ ions are both monovalent cations capable of adsorbing onto

monovalent anionic sites such as the β- and y-carboxyl groups of the cell proteins. Theoretical calculations, made on the basis of the fundamental physical constants of the K^+ and Na+ ions, show that when the electron density of the anionic groups (i.e., c-value) changes, the preference of the site may change from a preponderant preference'for K^+ over Na^+ ion to another in which this preference is diminished or even reversed (ref. 7, chapter 4). In normal resting muscle cells, the c-value is such that K^+ ion is preferentially adsorbed by a large factor. Thus in spite of the large concentration difference between Na+ (100 mM) and K^+ ion (2.5 mM) in the normal cellular environment, the greatest number of the intracellular monovalent anionic sites are occupied by K^+ ion. In the resting muscle cells more than 99% of the intracellular K^+ ion exists in an adsorbed state and half of the intracellular Na+ ion is adsorbed, the rest being interstitial in the cell water.[8,13]

Rate-Limiting Steps in *Na^+-ion Efflux*

One may next ask the question: What is the rate-limiting step in a normal Na^+-ion efflux curve? In the past it was accepted that the only part of the Na^+-ion efflux curve emanating from the cell was the straight-line segment appearing after about 20 min of washing (25°C) (Figs. 2, 3). The initial rapidly exchanging fraction amounting to some 25 to 40% of the total tissue Na+ ion was attributed to extraneous elements.[3,18,19,24] Using four independent new methods,[25–28] however, it has been more recently shown that the extracellular space and connective tissue contribution do not constitute the 25 to 40% figure often assigned in recent literature but total closer to 10%. To disregard the initial fast efflux is, therefore, no longer reasonable.[29] As mentioned above, there is also strong evidence that an important fraction of intracellular Na+ ion, in resting cells and in cells previously equilibrated in a K-poor medium, is absorbed. Therefore it is imperative to re-evaluate the conventional view that the flat part of the curve represents rate-limitation by membrane permeability.

Extensive investigation[17] of single- and multiple-fiber preparations has in fact led to a contrary conclusion. The evidence indicates that a significant part of the initial rapid exchange, although conventionally ascribed to extracellular space, also originates within the cell. Indeed, it represents the efflux of the free interstitial Na+ ion in the cell water. However, the slower part of the curve, conventionally regarded as rate-limited by membrane permeability, is rate-limited chiefly by desorption from intracellular adsorption sites. Ling and Walton, taking advantage of a newly reported way to remove extracellular fluid,[26] showed quite clearly the intracellular origin of the fast as well as slow fractions of Na^+.[29]

In the light of this modern interpretation, a progressive flattening of the straight-line fraction with increasing duration of preincubation in ^{22}Na-containing K-free solution, as seen in Figs. 5 and 6, indicates a progressive slowdown of the desorption rate.

Cooperative Adsorption and Desorption

According to the association-induction hypothesis, cell proteins are capable of cooperative interaction—that is, adsorption on one site of a specific solute may influence the affinity of the near neighboring sites for the same solution. Such *auto*-cooperative interaction will manifest itself when two solutes with different adsorption energy are both present in the environment to compete for the same sites.[7,13,15,30] If the interaction is autocooperative, the presence of a solute on one site enhances the relative affinity of the two nearest neighboring sites for the same species of solute. On the other hand, if the adsorption of a solute diminishes the relative affinity of the neighboring sites for the same species of solute, the interaction is referred to as heterocooperative. Biologically speaking, the more important kind of interaction is of the autocooperative type.[7]

The large disparity in the adsorption energies of K^+ and Na+ ions suggests that an autocooperative type of adsorption isotherm might be seen, and this has indeed been found to be the case.[13–15] The data show a nearest-neighbor interaction $(-\gamma/2)$ of 0.53 ± 0.04 kcal/mole and an intrinsic standard free energy $(-\Delta F_{Na-K}^{\infty})$ of the K-Na equilibrium of 2.94 kcal/mole. This means that if the sites are all occupied by Na+ ion rather than by K^+ ion, the relative affinity of the sites for Na+ ion is larger by a factor of $2 \times 0.53 = 1.1$ kcal/mole. Our findings in this regard have been confirmed repeatedly—not least by Jones and Karreman,[14] who investigated the K-Na ion distribution in canine carotid arteries. The $-\gamma/2$ and $-\Delta F_{Na-K}^{\infty}$ are, respectively, 0.6 and 3.5 kcal/mole.

With this background information in mind, let us examine some of the outstanding features of the present data.

1. Indifference of labeled *Na^+-ion* efflux from normal muscle to the K+-ion concentration in the washing solution (Fig. 2). Pre-incubation of muscle in Na^+-ion labeled, K+-free solute has a twofold function: (a) removal of K^+ ions from the adsorption sites and (b) replacement of the removed K^+ ions with labeled Na^+ ions found in the intracellular (and extracellular) water. Exposure of labeled normal muscle to a K+-free Ringer after 40 min of washing in a normal Ringer's (Fig. 2) serves the first function. However, it does not serve the second function because after 40 min of washing, only a negligible amount of labeled Na+ ion can be found in the intracellular water. On the other hand, when ^{22}Na-loaded muscle is washed in a K+-ion free solution right from the start, the concentration of labeled Na+ ion in the cell water is, for a brief period, substantial. In consequence, a fraction of the labeled Na^+ ion does become cooperatively adsorbed. This fraction was revealed when the more rapidly exchanging fraction had been washed away (Fig. 3). But it is when the entire K^+, Na+ ion-exchange process occurs in an environment where all the Na+ ion in the cell water and in the external medium is labeled that the cooperative displacement by labeled Na+ can occur with a marked slowing of the subsequent Na+ ion efflux, as witnessed in the data of Figs. 3, 4, 5, and 6.

2. Increase of $t_{1/2}$ with increase of pre-incubation time in K-free Ringer's. In a simplified model one may conceive of one type of adsorption site. (This model is undoubtedly oversimplified but there is evidence that it is not too far from the truth.[14,15]) In such case there would be three different values for the adsorption energy of a Na^+ ion, each corresponding to that of the central Na^+ ion in the triads KNaK, KNaNa, and NaNaNa. From the above-mentioned energies measured, the adsorption energy of the central Na^+ ion increases from left to right, in a stepwise manner, by 0.5 kcal/mole. The lowest adsorption energy is that of Na^+ ion in a muscle in the K-state (as in a normal resting muscle); the rate of exchange desorption is faster in this state. The highest adsorption energy corresponds to the Na^+ ion in a muscle in the Na state when most ^{22}Na is flanked by other labeled and non-labeled Na^+. To desorb, a labeled Na^+ must overcome the activation energy that contains this adsorption energy term. The rate of desorption of labeled Na^+ in a muscle in the Na state is naturally slower than that of labeled Na^+ in a muscle in the K-state.

Thus in a K-free environment the K-state is gradually shifted to the Na^+ ion state. With this shift, adsorbed Na^+ ion with Na^+ ion neighbors increases until eventually nearly all K^+ ion is replaced by Na^+ ion, and the rate of Na^+-ion exchange—hence of Na^+-ion efflux rate—reaches a minimum and $t_{1/2}$ a maximum.

The events described in Fig. 6 in fact represent a kinetic event occurring in an autocooperative assembly, or, more properly, a stochastic process. Karreman has presented a theoretical treatment applicable to K vs. Na cooperative adsorption in living cells.[31] Negendank demonstrated good agreement between that theory and experimental data of the time course of the regaining of K^+ in K+-depleted human lymphocytes.[32]

Although unavailable as yet are sufficiently extensive data on the reverse $K^+ \rightarrow Na^+$ exchange to merit a theoretical analysis, there is little doubt that the increase of Na^+ belonging to the slow fraction as well as the progressive increase of $t_{1/2}$ of this fraction (Fig. 6) have the same underlying mechanism.

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REFERENCES

1. A. L. Hodgkin and R. D. Keynes, "Active transport in giant axons: *Sepia Loligo*," *J. Physiol. (London), 128,* 28 (1955).
2. E. J. Harris and M. Maizels, "The permeability of human erythrocytes to sodium," *ibid,* 113, 506 (1951).
3. R. D. Keynes, "The ionic fluxes in frog muscle," *Proc. R. Soc. London, Ser. B., 142,* 359 (1954).

4. J. M. Glynn, "Linked sodium and potassium movements in human red cells," *J. Physiol.*, 126, 35 (1954).
5. T. J. Shaw, "Sodium and potassium movements in red cells," *Ph.D. Thesis,* Cambridge University, 1954.
6. H. H. Ussing, "Ion transport across biological membranes," in *Ion Transport Across Membranes, H.* T. Clarke and D. Nachmansohn, Eds., Academic Press, New York, 1954.
7. G. N. Ling, *A Physical Theory of the Living State: The Association-Induction Hypothesis,* Blaisdell Publishing, Waltham, Massachusetts, 1962.
8. ———, "The association-induction hypothesis," *Tex. Rep. Biol. Med., 22,* 244 (1964).
9. ———, "The physical state of water in living cell and model systems," *Ann.* NY *Acad. Sci.,* 125,401 (1965).
10. G. N. Ling and M. M. Ochsenfeld, "Confirmation of 'universality rule' in solute distributions: Studies of simultaneous efflux of Na^+ and D-arabinose from single frog eggs living, dying, and dead," *Physiol. Chem. Phys., 9,* 405 (1977).
11. ———, "Studies on ion accumulation in muscle cells," J. *Gen. Physiol., 49,* 819 (1966).
12. J. A. Hinke, J. P. Caillé and D. C. Gayton, "Distribution and state of monovalent ions in skeletal muscle based on ion electrode, isotope and diffusion analyses," *Ann.* NY *Acad. Sci., 204,* 274 (1973).
13. G. N. Ling, "All-or-none adsorption by living cells and model protein-water systems: Discussion of the problems of 'permease-induction' and determination of secondary and tertiary structures of proteins," *Fed. Pioc.,* 25, 958 (1966).
14. A. W. Jones and G. Karremann, "Potassium accumulation and permeation in the canine carotid artery," *Biophys.* J., 9, 910 (1969).
15. G. N. Ling and G. Bohr, "Studies on ion distribution in living cells. II. Cooperative interaction between intracellular K^+ and Na^+ ions," *ibid., 10,* 519 (1970).
16. ———, "Studies on ionic distribution in living cells. I. Long-term preservation of isolated frog muscles," *Physiol. Chem. Phys.,* 1, 591 (1969).
17. G. N. Ling, "Studies on ion permeability. I. What determines the rate of Na^+ ion efflux from frog muscle cells?," *ibid,* 2, 242 (1970).
18. L. J. Mullins and A. S. Frumento, "The concentration dependence of sodium efflux from muscle," J. *Gen. Physiol., 46,* 629 (1963).
19. E. J. Harris, "The dependence of efflux of sodium from frog muscle on internal sodium and external potassium," J. *Physiol. (London), 177,* 355 (1965).
20. R. B. Dean, "Theories of electrolyte equilibrium in muscles," *Biol. Symp.,* 3, 331 (1941).
21. G. N. Ling, "The physical state of water in biological systems," *Food Technol.,* 22, 1254 (1968).
22. ———, "The role of phosphate in the maintenance of the resting potential and selective ionic accumulation in frog muscle cells," in *Phosphorous Metabolism,* Vol. II, W. D. McElroy and B. Glass, Eds., Johns Hopkins University Press, Baltimore, 1952.
23. ———, "A new model for the living cell: A summary of the theory and recent experimental evidence in its support," *Int. Rev. Cytol., 26,* 1 (1969).
24. J. A. Johnson, "Kinetics of release of radioactive sodium, sulfate and sucrose from a frog sartorius muscle," *Am. J. Physiol.,* 181, 263 (1955).
25. G. N. Ling and M. H. Kromash, "The extracellular space of voluntary muscle tissues," *J. Gen. Physiol.,* 50, 677 (1967).
26. G .N. Ling, M. C. Neville, S. Will and P. Shannon, "Studies on insuline action. II. The extracellular space of frog muscle: Demonstration of D-mannitol and sucrose entry into isolated single muscle fibers and intact muscles," *Physiol. Chem. Phys.,* 1, 85 (1969).
27. G. N. Ling, "Studies of ion permeability. III. Diffusion of Br^- ion in the extracellular space of frog muscles," *ibid, 4,* 199 (1972).
28. ———, "A simple rapid method for the quantitative separation of the extracellular fluid in frog muscle," *ibid,* 7, 215 (1975).
29. G. N. Ling, C. Miller and M. M. Ochsenfeld, "The physical state of solutes and water in

living cells according to the association-induction hypothesis," *Ann.* NY *Acad.* Sci., 204, 6 **(1973).**
30. G. N. Ling and M. M. Ochsenfeld, "Control of cooperative adsorption of solutes and water in living cells by hormones, drugs and metabolic products," ibid, **325.**
31. G. Karremann, "Stochastic treatment of cooperative specific adsorption," Bull. *Math.* Biol., **33,483 (1971).**
32. W. Negendank and G. Karremann, "Exchange by human lymphocytes: Prediction of the cooperative adsorption model," J. Cell. *Physiol.,* in press.

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