Na+ AND K+ LEVELS IN LIVING CELLS: DO THEY DEPEND ON THE RATE OF OUTWARD TRANSPORT OF Na+?

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• ***At 25°C, frog sartorius muscles rapidly gained Na+ and lost K+ in iodoacetamide and pure nitrogen. Beginning at normal levels, the concentrations of these ions in the cells reached those in the surrounding Ringer solution in 140 min. Yet during that time the Na+ efflux rate showed no sign of the slowing down demanded by Na-pump theory. The data support the view that maintenance and alterations of Na+ levels in frog muscle cells reflect adsorption on protein sites and the solubility property of bulk phase water and are independent of the rate at which Na+ leaves the cell surface.***

Virtually all living cells maintain within themselves a low concentration of Na+ and a high concentration of K+. According to the pump theory, this asymmetry in ion distribution reflects continuous activities of postulated pumps located in the cell membrane.[1-3] In this view, the outward flux rate of Na+ from the cell is to a large extent determined by the rate of outward pumping. The observation that in frog muscles and other tissues exposed to cardiac glycosides such as ouabain the Na+ rate is reduced (by 90%[4]) has been vigorously argued as evidence affirming the pump theory[5,6] because *(1)* ouabain inhibits an ATPase that can be isolated from certain types of cell preparations containing cell membranes, and (2) the uninhibited activity of this ATPase is considered essential for the energization and normal functioning of the Na pump. However, totally different interpretations of these observations also have been proposed.[4,7-9]

A more unequivocal way to block the energy supply to the postulated pump is to apply well-known metabolic poisons. In fact, experiments designed to study the effect of these poisons on Na+ efflux have been reported.[10,11] Contrary to expectation, when these experiments were carried out at O°C the efflux rate of the fraction of labeled Na+ sensitive to ouabain,* as well as the levels of K+ and Na+ in frog

* **It is widely accepted that the "slow fraction" of Na+ efflux with a half-time exchange ($t_{1/2}$) of 20 to 40 min at room temperature in normal frog muscle represents the rate of membrane-limited intracellular-extracellular exchange of Na+. Considerable evidence now exists suggesting that a faster fraction with a $t_{1/2}$ of 2 to 4 min more accurately represents the intracellular-extracellular exchange rate. Like the slow fraction, however, the fast fraction is not slowed down by metabolic poisons, as we reported earlier in a different context.'"**

muscles, remained for many hours unaffected by the simultaneous suppression of respiration (with cyanide) and glycolysis (with sodium iodoacetate). Keynes and Maisel[13] and Conway et al.[14] confirmed the essence of this finding. In addition to the effect of sodium iodoacetate, Keynes and Maisel studied that of another metabolic poison, 2, 4-dinitrophenol, with similar results.

To explain these surprising findings, it was suggested that insensitivity of the Na^+ efflux rate to metabolic arrest might reflect the large energy store in the form of ATP and creatine phosphate in muscle cells.[13] Within the confines of the pump theory, a consistent explanation of this sort implies the following two assumptions: *(1)* that as long as the Na^+ efflux remains normal, the levels of Na^+ and K^+ in the cell will also remain normal, and (2) that after these energy reserves are used up, the Na^+ efflux rate will eventually slow down and, with that slow-down, the levels of Na^+ and K^+ in the cell will gradually approach those in the surrounding medium. But no experimental evidence has been adduced to support either of those assumptions. Furthermore, we recently reinvestigated the problem in some detail and found experimental data (to be presented below) contradicting both assumptions.

MATERIALS AND METHODS

All experiments were performed on the isolated sartorius muscles of North American leopard frogs (*Rana pipiens pipiens*, Schreber). Chemicals used were of reagent grade. The Ringer phosphate solution used for washing contained NaCl, 104.7 mM; KCl, 2.5 mM; $CaCl_2$, 1.0 mM; $MgSO_4$, 1.2 mM; $NaHCO_3$, 6.6 mM; NaH_2PO_4, 2.0 mM; and Na_2HPO_4, 1.2 mM. The Ringer-GIB medium was the same as that described by Ling and Bohr.[15]

^{22}Na was from International Chemical and Nuclear Corporation, Irvine, Calif., iodoacetamide from Sigma Chemical Co., St. Louis, Mo., N_2 and 95% N_2 + 5% CO_2 from Air Products, Allentown, Pa., were further purified from oxygen by passage through a heated tower of activated copper. The labeled Na^+ efflux was followed by washing the labeled muscles in 10 ml portions of Ringer phosphate solution while the solutions were bubbled with moistened air or with purified 95% N_2 + 5% CO_2, and by assaying the radioactivity of each of the washing solutions and the radioactivity remaining in the muscle at the conclusion of the experiment.[16] ^{22}Na was assayed on a Nuclear Chicago automatic γ-scintillation counter. The methods of extraction and assay of the total Na and K on a flame photometer were essentially similar to those described earlier.[15]

RESULTS

Figure 1 shows that at 0°C, iodoacetamide (IAA) and pure nitrogen had

relatively little effect on the K^+ and Na^+ contents of frog muscles. However, changes in these ionic contents occurred promptly after the temperature was raised to 25°C. Indeed, within 140 min following the temperature change, K^+ in the cells fell from a normal level of about 100 mmoles/kg to that of the surrounding medium (2.5 mM), while Na^+ rose from a normal level of 20 mmoles/kg to a point where it first equaled and then exceeded that in the medium (100 mM).

In Fig. 2 is plotted the observed time course of the Na^+ efflux rate from normal muscles washed in a normal Ringer solution and from their pairs washed in a similar Ringer solution containing IAA and pure nitrogen. The poisoned muscles studied in

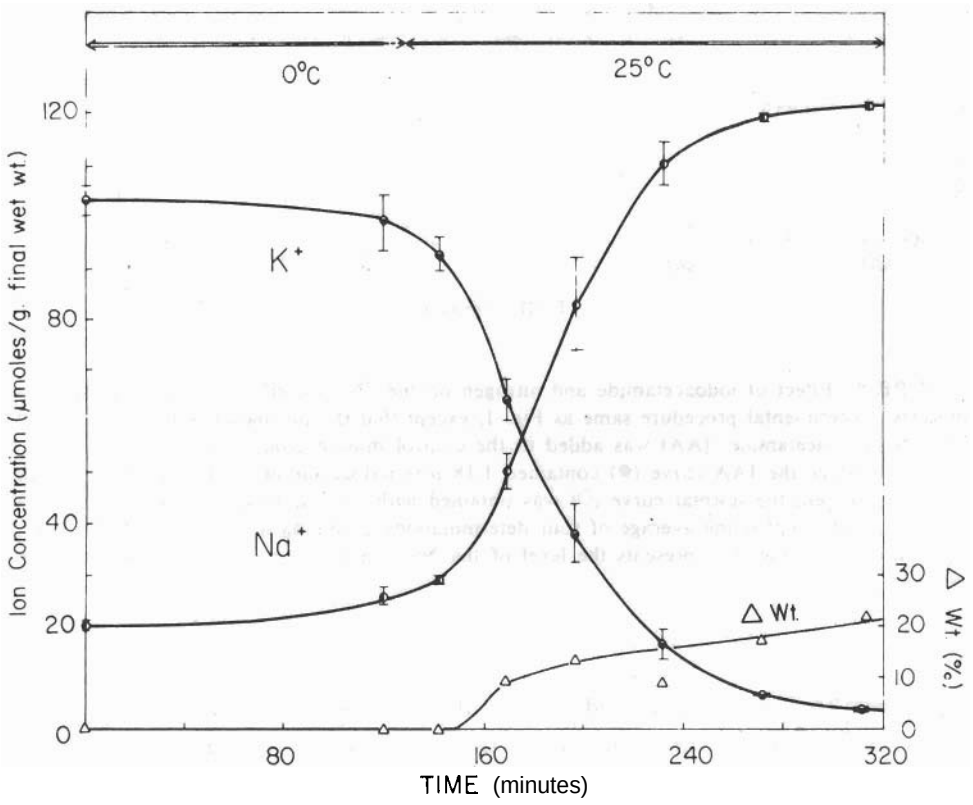

FIGURE 1. Time course of the effect of iodoacetamide and nitrogen on the K and Na' contents of frog sartorius muscles. Muscles were incubated in Ringer-GI9 solution containing 100 mM NaCl at 0°C. After 19 h, iodoacetamide (final concentration, 1.18 mM) was added to the solution and incubation continued an additional 2 h in purified nitrogen. The muscles were then washed with a Ringer phosphate solution containing 1.18 mM iodoacetamide (see arrow) and bubbled with purified nitrogen. During the first 10 min of washing the temperature was 0°C; throughout the remaining wash time the temperature was 25°C. Each point on the K^+ (◒) and Na' (◐) curves represents the average of four muscles ± standard error. The lowest curve, marked ΔWt., represents the change in wet weight of the muscles with time.

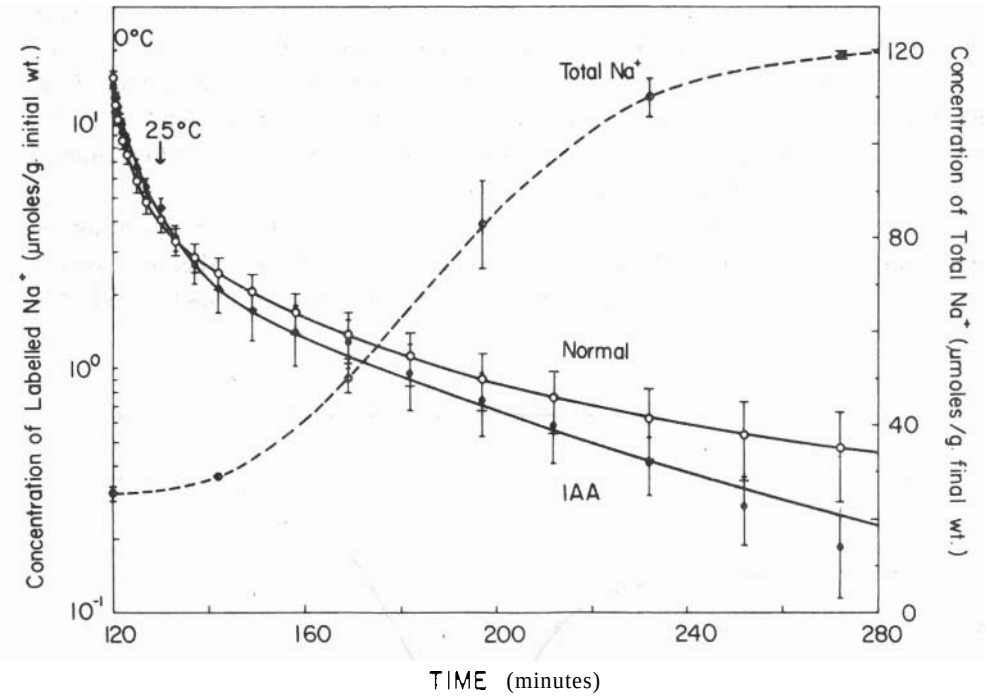

FIGURE 2. Effect of iodoacetamide and nitrogen on the ^{22}Na-ion efflux of the frog sartorius muscles. Experimental procedure same as Fig. 1, except that the incubation solution contained ^{22}Na. No iodoacetamide (IAA) was added to the control muscle group. The washing solution used to produce the IAA curve (●) contained 1.18 mM iodoacetamide and was bubbled with purified nitrogen; the normal curve (○) was obtained with normal Ringer phosphate bubbled with air. Each point is the average of four determinations ± standard error. The dashed line, reproduced from Fig. 1, represents the level of the Na+ ion in the muscle at equivalent time periods.

Fig. 2 were treated in a manner identical to that used in the experiment described in Fig. 1. To facilitate comparison, part of the data of Fig. 1 (showing the changing level of total cell Na+) is reproduced in Fig. 2 (dashed curve).

The data of Fig. 2 confirm earlier reports mentioned above, [10,13,14] showing quite clearly that from the beginning to the end of the experiment the rate of Na+ efflux of the poisoned muscles gave no indication of slowing down. Indeed, the Na^+ efflux was considerably faster than that of the normal controls since the poisoned muscles, but not the controls, rapidly gained Na^+. (The slope of the efflux curves as shown in Fig. 2 represents the rate constant of exchange of labeled Na^+. The Na+ efflux rate is calculated as the product of the rate constant and the free Na+ concentration in the cell. In the poisoned muscles but not in their normal controls, the free Na+ level

in the cell, and hence the Na+ efflux rate, increased rapidly with time. Similar results were obtained when iodosobenzoate plus nitrogen was used.)

DISCUSSION

Now, if levels of K^+ and Na+ in cells are maintained by pumps, there is little question that such pumps would have been rendered non-functional by poisons that completely destroyed the normal asymmetry of K+ and Na+ distribution in 140 min. As mentioned above, the pump theory demands a reduction in the rate of Na+ efflux while the muscle is gaining Na^+. Yet the data show no such predicted reduction in the Na+ efflux rate.

The present demonstration of a complete dissociation of the Na+ efflux rate on the one hand and the levels of Na^+ and K^+ in the cells on the other puts in a different light the earlier reported slow-down of Na+ efflux in the giant nerve axons of *Loligo* following the application of metabolic poisons.[17,18] While it is not possible to reconcile the results of the present muscle experiment with the Na-pump theory, it is readily possible to reconcile the results of both the muscle and nerve experiments with the association-induction hypothesis. Thus, it is well known that K^+ efflux from living cells is as a rule many times slower than Na+ efflux. According to the association-induction hypothesis, the slower rate of K^+ efflux reflects the preference of surface anionic sites for K+ (over Na^+) and the greater difficulty of the preferentially adsorbed K^+ to dissociate from the surface sites into the surrounding medium. Depletion of ATP, like the addition of ouabain,[4,7] causes in nerve tissue an increase in the c-value and a gain of the relative affinity of the surface anionic sites for Na^+,[11,19] which then assumes a behavior close to that of K^+ in normal cells and shows a decreased rate of efflux. Apparently surface adsorption of Na+ in dying muscles is much weaker.

The unchanging rate of Na^+ efflux over the period of time exhibiting the most rapid gain of total Na+ and loss of K+ (150 to 200 min; see Fig. 2) also shows that in the context of the pump theory, poisoning did not cause gain of intracellular Na^+ and loss of intracellular K^+ by producing a leakage of the cell membrane. Were it otherwise, there should have been a rapid increase in the outward leakage of labeled Na+ during the experiments, whereas in fact there was none.

The total dissociation of the rate of Na+ efflux from the maintenance of Na^+ (and K+) levels in the cell adds experimental evidence against the Na-pump theory to that presented in 1962, which indicated that the Na pump alone, under certain specified conditions, would consume more energy than the cell has at its disposal by as much as 3,000%.[11,20] (Recent attempts by Freedman[21] to postulate a reduction in energy requirement for the Na pump by means of a relocation of virtually all cell Na+ to the sarcoplasm reticulum have been shown experimentally to have no valid-

ity.[22]) Still another refutation presented in 1973 showed that in an effectively membrane-pumpless cell preparation, ouabain-sensitive Na+ exclusion persists.[23]

CONCLUSION

While contradicting the membrane pump theory, the data presented here lend support to the association-induction hypothesis, according to which K^+ and Na+ levels in cells represent a metastable equilibrium phenomenon reflecting the electronic conformation of the cell proteins and the physical state of the bulk of cell water.

The key effect of metabolic poisons is to block the resynthesis of ATP. Without the allosteric control provided by the cardinal adsorbent, ATP, the intracellular proteins revert to different electronic and steric conformations in which the capacity for preferential K^+ adsorption and for multilayer water adsorption is lost or profoundly altered, as confirmed recently by findings of profound change in water retention in living cells.[24]

Thus in the association-induction model, the rise in intracellular Na^+ concentration in the face of metabolic arrest reflects an "enlargement" of the "Na^+-dissolving space" in the depolarization of cell water.[25–27] No causal relation is expected between the Na^+ concentration in the cell and the rate at which Na+ leaves the cell surface; as this report shows, none was found.

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