# SIMULTANEOUS EFFLUX OF $K^+$ AND $Na^+$ FROM FROG SARTORIUS MUSCLE FREED OF EXTRACELLULAR FLUIDS: EVIDENCE FOR RAPIDLY EXCHANGING $Na^+$ FROM THE CELLS

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SUMMARY

After removal of radioactivity trapped in the extracellular space and correcting for the contribution of connective tissue elements, the $K^+$-efflux curve of frog sartorius muscles becomes a perfect straight line in a semilogarithmic plot. The simultaneously recorded $Na^+$-efflux curve from the same muscles remains strongly curved, and can be resolved into a slow fraction (which conventionally has been regarded as representing the entire cell $Na^+$) and at least one fast fraction. The fast fraction of $Na^+$ could not have originated from a sarcoplasmic reticulum or any other extracellular space extensions; otherwise a similar fast fraction should exist for $K^+$. The data agree with the interpretation that it is the fast fraction that is rate limiting by cell permeability and the slow fraction by desorption from intracellular adsorption sites.

## INTRODUCTION

When Levi and Ussing[1] first studied labeled $Na^+$ efflux from frog sartorius muscles, they recognized two fractions of labeled $Na^+$ from the tissue, one fast and one slow. They considered the slow fraction with a half time exchange ($t_{½}$) of about 30 minutes as representing $Na^+$ from the cell and the faster fraction as representing $Na^+$ from the extracellular space.

Levi and Ussing's original assignment has become widely accepted. Indeed, reading recent literature on this and related subjects, one cannot escape the impression that its validity must have been long proven. The truth is different. Evidence exists (ref. 2, p. 293; ref. 3, p. 838; ref. 4; ref. 5, p. 19) which has pointed to the need for further study on the subject. The results of such an undertaking are reported in this communication.

## MATERIALS AND METHODS

Isolated sartorius muscles of Northern American leopard frogs (Rana *pipiens pipiens*, Schreber) were used in all experiments. $Na^{22}$ was obtained from International Chemical and Nuclear Corporation (ICN) (Irvine, California); $K^{42}$ from ICN and from Cambridge Nuclear Pharmaceutical Corporation (Princeton, New Jersey).

For isotope loading, isolated sartorius muscles were incubated in a sterile Ringer-GIB medium[6] in equilibrium with 95% $O_2$ + 5% $CO_2$.

To remove fluids from the extracellular space, the muscles were centrifuged for 4 min at 1000 g in a hermetically sealed packet according to the procedure described by Ling and Walton.[7]

In efflux studies, isotope-loaded muscles, with or without prior centrifugation, were washed in successive portions of nonradioactive normal Ringer-phosphate solution which was vigorously stirred with air. The radioactivity of the washing solutions as well as that remaining in the muscle at the conclusion of the experiments was assayed on a Nuclear Chicago automatic y-scintillation counter. The normal Ringer-phosphate solution used contained: NaCl, 104.7 mM; KCI. 2.5 mM; $CaCl_2$, 1.0 mM; $MgSO_4$, 1.2 mM; $NaHCO_3$, 6.6 mM; $NaH_2PO_4$, 2.0 mM; $Na_2HPO_4$, 1.2 mM.

To correct for the contribution to the ionic effluxes of "connective tissue elements" (loose connective tissue, small blood vessels, small nerves, etc.) connective tissues from regions in the vicinity of the sartorius muscle and from the legs were isolated and treated in an identical manner as the sartorius muscles. The efflux curves of these connective tissues obtained were then used to make corrections for the muscle effluxes.

## RESULTS

### *Simultaneous $Na^+$- and $K^+$-Efflux Curves*

Using double-labeling techniques, we studied the simultaneous efflux of both labeled $K^-$ and labeled $Na^+$ from frog sartorius muscles. Figure 1 shows four sets of data presented on a semilogarithmic scale without any correction. The different appearance of the semilogarithmic plots of the $Na^+$- and $K^+$-efflux curves, reported previously for effluxes made on different muscles[4,5] have now been observed in the same muscle at the same time; a pronounced curvature in the $Na^c$-efflux curve vis-a-vis a more-or-less straight $K^-$-efflux curve.

In the experiments described in Figure 1, the muscles were equilibrated overnight in a Ringer-phosphate solution containing labeled $Na^+$. $K^{42}$ was introduced into the Ringer solution approximately 33 min before the removal of the muscle from the incubation solution. The short exposure to labeled $K^+$ was designed to minimize the total labeled $K^+$ accumulated in the muscle cells, since a high level of labeled $K^+$ in the cells tends to

obscure the labeled $K^+$ in the extracellular space.

*Corrected Simultaneous $Na^+$- and $K^+$-Efflux Curves*

Both $K^+$ and $Na^+$ curves as presented in Figure 1 reflect not only changes in labeled ion concentration in muscle cells but also, to some extent, changes in the extracellular space and the "connective tissue elements."

To reveal accurately the efflux characteristics of muscle cells themselves, two steps had to be taken: eliminate radioactive ions in the extracellular fluid by centrifugation and to subtract the contribution of labeled ion efflux of the connective tissue elements.

(1) Removal of Extracellular Fluids: Four sets of simultaneously measured $K^+$- and $Na^+$-efflux curves from centrifuged muscle are shown in Figure 2. The heavy solid lines going through the experimental points have not been corrected and are, therefore, equivalent to similar curves shown in Figure 1. The only difference lies in the fact that the extracellular space fluids in the muscles in Figure 2 were removed by centrifugation before washing began. At first glance, the curves in Figures 1 and 2 were hardly distinguishable: the near-perfect straight line of the $K^+$-efflux curve and the presence of both fast and slow fractions in the $Na^+$ curve remained unchanged.

Thus, the original determination that the entire fast fraction in the $Na^+$-efflux curve is extracellular in origin is clearly not correct.

It should be pointed out, however, that the ordinate in Figure 1 refers to micromoles of ions per gram of fresh tissue and that this fresh tissue weight included the weight of the extracellular fluids. The ordinate in Figure 2, on the other hand, refers to micromoles of ions per gram of fresh tissue freed of extracellular fluid. The difference in weight basis used accounts for the nearly equal numerical values of the initial $Na^+$ concentration in muscles with and without their extracellular fluid.

(2) Correction for Connective Tissue Element Contributions: The corrections for the contribution of connective tissue elements to efflux profiles are more complicated because (a) the weight percentage of connective tissues in individual sartorius muscle exhibits considerable variation, even though the average weight percentage is fairly constant, and (b) the composition of the connective tissue elements in the sartorius muscles and in the loose connective tissues used as a model may not be exactly the same.

(a) Weight Percentage of Connective Tissue: In previous publications, it was shown that the average weight of connective tissues is 9.1% of the total fresh muscle tissue as determined by the total collagen and elastin contents (ref. 2, p. 219), and 8.2% by the $Br^-$-efflux method.[1] In these cases, both the fresh weights of the muscle tissues and those of the connective tissues refer to fresh weights after blotting, using a standard procedure described by Ling and Bohr.[6] Centrifugation at 1000 g for 4 min removes on the average about 9% of the fresh weight of the muscle tissue[7] but 41.3% ± 0.86% of the weight of the connective tissues (Ling & Ochsenfeld, unpublished results). Thus, in terms of centri-

fuged tissues, the average weight percentage of connective tissue is no longer 9% but only

$$\frac{9}{100} \times \frac{(1-0.41)}{(1-0.09)} = 5.9\%$$

(b) Correction for Connective Tissue Elements in a Frog Sartorius Muscle: Clearly revealed in a muscle whose cellular proteins have been removed by prior alkaline digestion[9] (also, ref. 2, p. 210) are the collagen of the fascia covering the outer surface of the muscle and the extensive tubular system of the sarcolemma. In addition, the connective tissue elements also contained small nerve fibers, small blood vessels, blood cells occasionally trapped in the blood vessels, fibroblasts, and small amounts of other cellular elements.

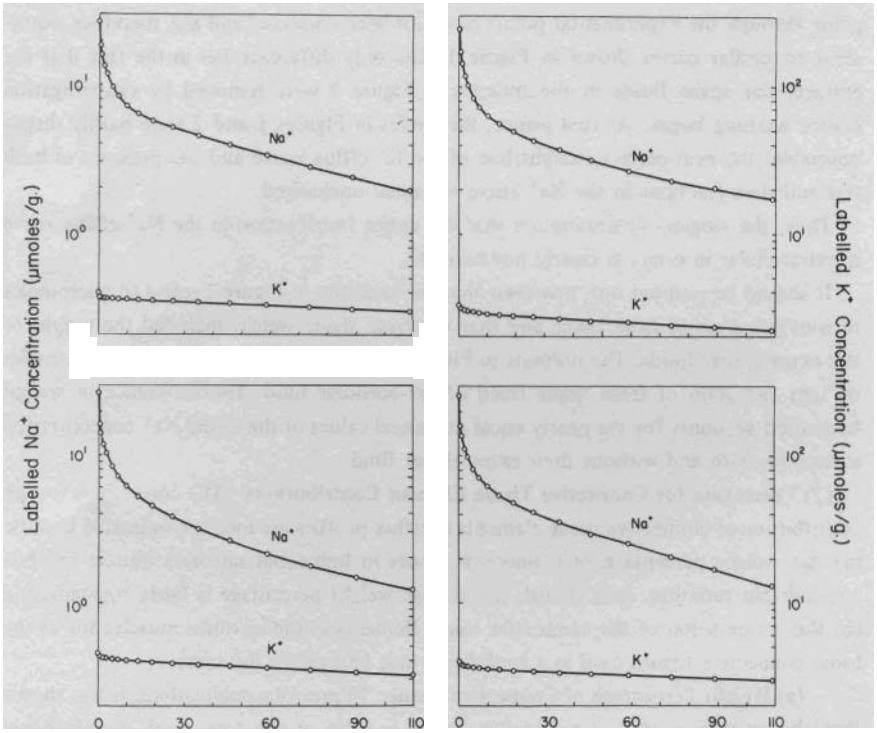


Figure 1. The time course of simultaneous $Na^+$-ion and $K^+$-ion efflux from frog sartorius muscles. All muscles were incubated overnight at 25°C with labeled $Na^+$. In A, labeled $K^+$ was added to the incubation for the final 33 minutes of incubation; in B, 25 minutes; in C, 35 minutes; and in D, 40 minutes. After blotting, the muscles were washed in successive tubes of Ringer-phosphate solution at 25°C. Ordinates are in μmoles per gram of fresh muscle tissue weight. Lines were drawn to fit best by visual inspection the experimental points, and no correction was made.

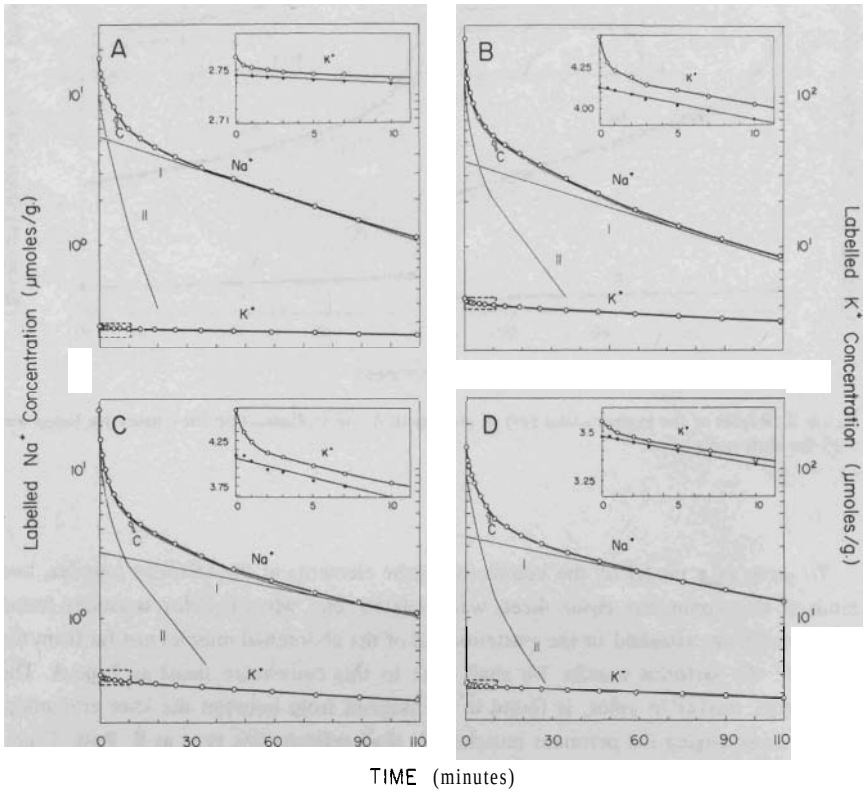


Figure 2. The time course of simultaneous $Na^+$-ion and $K^+$-ion efflux from centrifuged frog sartorius muscles. The muscle in A was incubated overnight at 25°C with labeled $Na^+$. Labeled $K^+$ was added to the solution for the final 33 minutes of incubation. The muscles in B, C, and D were incubated at 25°C with labeled $Na^+$ and labeled $K^+$ for 33 minutes. The muscles were exposed to the incubating solution for 10 minutes while wrapped in the centrifugation packet, which increases the total incubation time to 43 minutes. The muscles were centrifuged at 1000 g for 4 minutes before washing in successive tubes of Ringer-phosphate solution at 25°C. Correction for connective tissue was made on the basis of a composite curve of $Na^+$-ion efflux from similarly incubated connective tissues. The ordinate is in units of μmoles per gram of fresh muscle tissue. Line I was obtained from a 1.9% connective tissue correction in A, a 7.0% correction in B, a 6% correction in C. and a 1.9% correction in D. An enlargement of the first 10 minutes of $K^+$-ion efflux for each muscle is illustrated with the connective tissue correction.

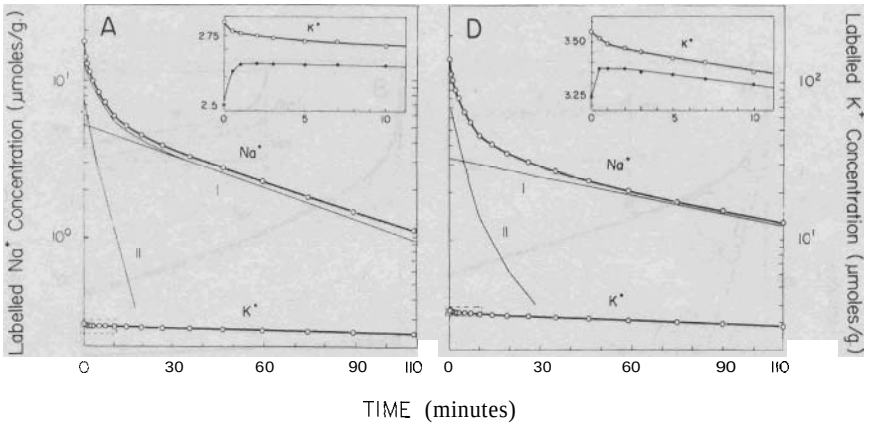


Figure 3. Replot of the experimental data of muscles in A and D. Correction for connective tissue was 7.0% for each curve.

To serve as a model of the connective tissue elements in the sartorius muscles, two kinds of thin connective tissue sheets were isolated: one, white in color, is usually found in skinned frogs attached to the posterior end of the abdominal muscles not far from the origin of the sartorius muscle. We shall refer to this connective tissue as Type **A.** The other type, darker in color, is found in the skinned frogs between the knee and ankle, loosely overhanging the peroneus muscles. We shall refer to this type as B. Both Types, **A** and B, resemble the connective tissue elements in the sartorius muscles.

Figures 4 and 5 show the simultaneous efflux curves of $K^+$ and $Na^+$ centrifuged Type **A** and B connective tissue, respectively. The $Na^+$-efflux curves from the two types are essentially the same but much more labeled $K^+$ was taken up by Type B than Type **A.** This larger uptake probably reflects the larger percentage of cellular elements, e.g., pigment cells which are present in Type B connective tissue only.

We used the $K^+$-efflux curve of the centrifuged muscle as a guideline to search for a maximum but reasonable connective tissue correction for each individual muscle. The guiding principle used was that, within the boundary of experimental error, the corrected labeled $K^+$ content of the tissue should at no time show an increase with time while the tissue was being washed in a solution devoid of labeled $K^+$. Once this maximum percentage of connective tissue elements for a particular muscle was found, it was used to correct for both the $K^+$- and $Na^+$-efflux curve of the same muscle.

The curves obtained from correcting for the connective tissue element contribution

based on Type A connective tissue effluxes (Fig. 4) are labeled with the capital letter C and are in all cases close to the uncorrected curves. Also shown in Figure 2 are two resolved fractions of the corrected $Na^+$-efflux curves. The percentage of connective tissues used in the corrections for the four experiments labeled A, B, C, and D are, respectively, 1.9%, 7%, 6%, and 1.9%. These figures were the maximum values that did not produce an unreasonable $K^{42}$ increase in the $K^+$-efflux curves. Indeed, what the correction did to the $K^+$-efflux curves was to make them *straight* lines in the semilogarithmic plot as shown in the insets of Figure 2.

*Yet the simultaneous $Na^+$-efflux curves from the same muscles freed of extracellular space fluids and after correction for the same connective tissue elements remain as strongly curved as ever and can be resolved into two fractions.* It should be pointed out that if the corrections were based on Type B connective tissue (Fig. 5), the alteration of the $Na^+$ curve would be less noticable, since Type B connective tissue contains more $K^+$ and therefore less $Na^+$ correction would be applied.

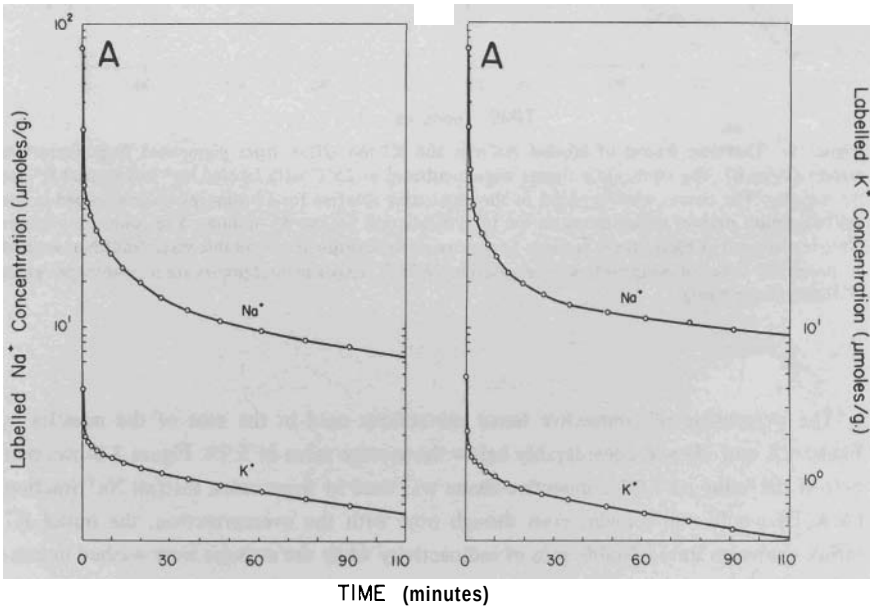


Figure 4. The time course of labeled $Na^+$-ion and $K^+$-ion efflux from white frog connective tissue (Type A). The connective tissues were incubated at 25°C with labeled $Na^+$ and labeled $K^+$ for 33 minutes. The muscles were exposed to the incubating solution for 10 minutes while wrapped in the centrifugation packet which increases the total incubation time to 43 minutes. The connective tissues were centrifuged at 1000 g for 4 minutes to remove centrifugational extractable water and then washed in successive tubes of Ringer-phosphate solution at 25°C. Experimental points are in μmoles per gram of fresh muscle tissue.

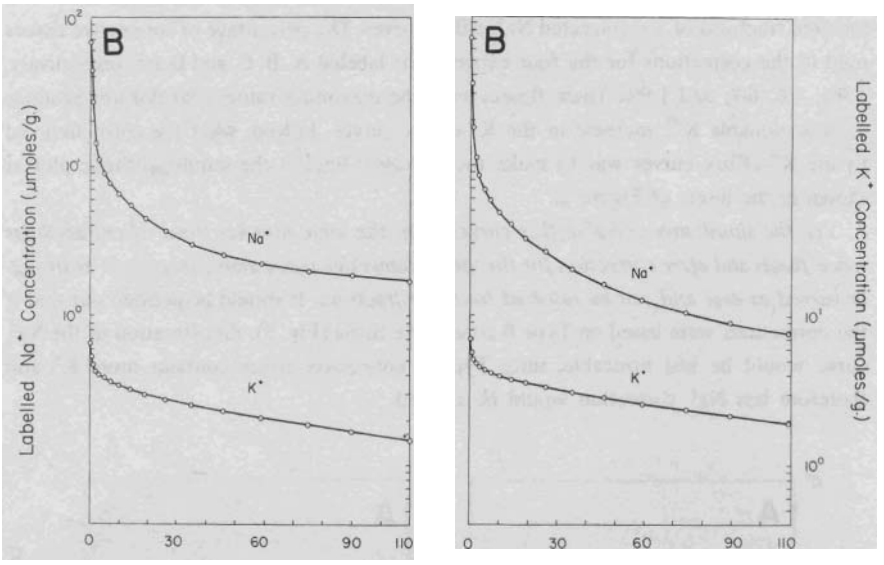


Figure 5. The time course of labeled $Na^+$-ion and $K^+$-ion efflux from pigmented frog connective tissues (Type B). The connective tissues were incubated at 25°C with labeled $Na^+$ and labeled $K^+$ for 33 minutes. The tissues were exposed to the incubating solution for 10 minutes while wrapped in the centrifugation packet, which increases the total incubation time to 43 minutes. The connective tissues were centrifuged at 1000 g for 4 minutes to remove centrifugational extractable water and then washed in successive tubes of Ringer-phosphate solution at 25°C. Experimental points are in μmoles per gram of fresh muscle tissue.

The percentage of connective tissue corrections used in the case of the muscles in Figure 2A and 2D was considerably below the average value of 5.9%. Figure 3 shows that even if the value of 7.0% connective tissue was used in these cases, the fast $Na^+$ fraction (A & D) would still remain, even though now with the overcorrection, the initial $K^+$ efflux shows an unreasonable gain of radioactivity while the muscles were washed in non-radioactive solutions.

In Tables 1-3 the experimental data are shown respectively for the simultaneous effluxes of $K^+$ and $Na^+$ from centrifuged and corrected muscles and centrifuged connective tissues. It is to be noted that in Part A of Table 1, $K^{42}$ loading time was short (43 min), while $Na^{22}$ loading time was lone (1000 min). In Part B, loading time for both isotopes was 43 min. Reducing the loading time for $Na^+$ does not materially affect the efflux curves.

Table 1. Data of $Na^{22}$ and $K^{42}$ from Centrifuged Frog Sartorius Muscles. All muscles in group A were incubated overnight at 25°C with labeled $Na^+$. Unlabeled $K^+$ was added for the final 33 minutes of incubation. The muscles in group B were incubated at 25°C with labeled $Na^+$ and labeled $K^+$ for 33 minutes. The muscles were exposed to the incubating solution for 10 minutes while wrapped in the centrifugation packet which increases the total incubation time to 43 minutes. The muscles were centrifuged at 1000 g for 4 minutes before washing in successive tubes of Ringer phosphate solution at 25°C.

| | $Na^{22}$ Efflux | | | | | | $K^{42}$ Efflux | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Slow Fraction | | Fast Fraction | | | | Main Fraction | |
| Experiment | Incubation Time | Total Labeled $Na^+$ | Connective Tissues | Conc. | $t_{1/2}$ | Conc. | Incubation Time | Total Labeled $K^+$ | Connective Tissue | Conc. | $t_{1/2}$ |
| | Min | μmoles/g | % | μmoles/g | Min | μmoles/g | Min | μmoles/g | % | μmoles/g | Min |
| Group A | | | | | | | | | | | |
| IV D2A | 1136 | 12.98 | 4.0 | 2.67 | 13.7 | 7.68 | 43 | 3.77 | 4.0 | 3.56 | 227 |
| IV D2B | 1136 | 15.78 | 3.0 | 2.38 | 32.9 | 11.45 | 43 | 2.87 | 3.0 | 2.71 | 262 |
| IV D2C | 1243 | 10.09 | 4.0 | 1.60 | 50.3 | 14.86 | 43 | 2.52 | 4.0 | 2.31 | 277 |
| IV D2B | 1356 | 20.74 | 4.7 | 3.11 | 19.8 | 14.53 | 43 | 2.42 | 4.7 | 2.18 | 217 |
| IV 2DF | 1356 | 16.47 | 4.0 | 2.64 | 15.4 | 11.22 | 43 | 2.69 | 4.0 | 2.48 | 157 |
| IV D3H | 2720 | 15.78 | 6.0 | 2.14 | 24.5 | 9.61 | 43 | 6.22 | 6.0 | 5.90 | 275 |
| V A29C | 1053 | 18.02 | 1.9 | 5.40 | 46.9 | 11.50 | 43 | 2.86 | 1.9 | 2.78 | 615 |
| V A29A | 1083 | 22.70 | 1.9 | 8.51 | 107.0 | 12.84 | 43 | 3.39 | 1.9 | 3.31 | 343 |
| Mean | | 17.70 | | 3.56 | 38.81 | 11.71 | | 3.34 | | 3.15 | 297 |
| ±S.E. | | ±1.10 | | ±.81 | ±10.88 | ±.85 | | ±.44 | | ±.43 | ±49 |
| Group B | | | | | | | | | | | |
| V A15A | 43 | 31.77 | 9.0 | 3.78 | 30.0 | 20.67 | 43 | 4.30 | 9.0 | 3.82 | 187 |
| V A15C | 43 | 26.18 | 7.0 | 4.05 | 47.3 | 17.01 | 43 | 4.77 | 7.0 | 4.44 | 268 |
| V A16C | 43 | 26.20 | 8.1 | 2.50 | 42.4 | 17.10 | 43 | 3.57 | 8.1 | 3.14 | 188 |
| V A16E | 43 | 14.30 | 1.9 | 3.55 | 75.0 | 9.44 | 43 | 3.62 | 1.9 | 3.53 | 364 |
| V A16G | 43 | 27.33 | 6.0 | 2.99 | 74.7 | 20.00 | 43 | 4.45 | 6.0 | 4.16 | 211 |
| Mean | | 25.16 | | 3.37 | 53.88 | 16.84 | | 4.14 | | 3.82 | 244 |
| ±S.E. | | ±2.90 | | ±.28 | ±9.01 | ±1.99 | | ±.24 | | ±.23 | ±34 |

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Table 2. Data of $Na^{22}$ and $K^{42}$ from Frog Sartorius Muscles. All muscles were incubated overnight at 25°C with labeled $Na^+$. Labeled $K^+$ was added for a final short period of incubation. After blotting, the muscles were washed in successive tubes of Ringer-phosphate solution at 25°C.

| | $Na^{22}$ Efflux | | | | | | $K^{42}$ Efflux | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Slow Fraction | | Fast Fraction | | | | Main Fraction | |
| Experiment | Incubation Time Min | Total Labeled $Na^+$ μmoles/g | Connective Tissues % | Conc. μmoles/g | $t_{1/2}$ Min | Conc. μmoles/g | Incubation Time Min | Total Labeled $K^+$ μmoles/g | Connective Tissues % | Conc. μmoles/g | $t_{1/2}$ Min |
| III LI8A | 1205 | 22.71 | 9.0 | 4.75 | 38.4 | 14.92 | 20 | 1.92 | * | 1.71 | 375 |
| III LI8B | 1220 | 29.43 | 9.0 | 4.58 | 48.5 | 21.68 | 35 | 4.56 | * | 4.20 | 275 |
| III LI8D | 1295 | 24.90 | 9.0 | 8.38 | 30.5 | 13.46 | 20 | 2.43 | * | 2.31 | 440 |
| III LI8E | 1230 | 29.07 | 9.0 | 6.91 | 34.9 | 19.02 | 45 | 4.93 | * | 4.72 | 323 |
| III LI9F | 2515 | 26.77 | 9.0 | 4.49 | 72.5 | 19.24 | 25 | 3.26 | * | 2.94 | 264 |
| III LI9G | 2530 | 28.32 | 9.0 | 5.86 | 45.7 | 19.45 | 40 | 4.05 | * | 3.65 | 343 |
| 111 LI9H | 2750 | 27.05 | 9.0 | 5.60 | 30.0 | 18.34 | 25 | 2.58 | * | 2.32 | 377 |
| III LI9J | 2795 | 26.99 | 9.0 | 6.75 | 22.0 | 16.99 | 70 | 5.80 | * | 5.50 | 342 |
| V A29B | 1053 | 29.62 | 9.0 | 7.26 | 53.0 | 19.33 | 33 | 3.78 | * | 3.57 | 425 |
| V A29D | 1083 | 26.38 | 9.0 | 7.12 | 84.0 | 16.15 | 33 | 3.84 | * | 3.69 | 440 |
| Mean ±S.E. | | 27.12 ±.68 | | 6.17 ±.42 | 46.0 t6.2 | 18.33 t.72 | | | | | 360 ±20 |

Table 3. Data of the $Na^{22}$ and $K^{42}$ Efflux from Frog Connective Tissue. The connective tissues in group A were incubated overnight at 25°C with labeled $Na^+$. Labeled $K^+$ was added for a final short period of incubation. After blotting, the muscles were washed in successive tubes of Ringer-phosphate solution at 25°C. The connective tissues in group B were incubated at 25°C with labeled $Na^+$ arid labeled $K^+$ for 33 minutes. The tissues were exposed to the incubating solution for 10 minutes while wrapped in the centrifugation packet, which increases the total incubation time to 43 minutes. The connective tissues were centrifuged at 1000 g for 4 minutes before washing in successive tubes of Ringer-phosphate solution at 25°C.

| Group | Symbol | Incubation Time minutes | Total Labeled $Na^+$ μmoles/g | Incubation Time minutes | Total Labeled $K^+$ μmoles/g |
|---|---|---|---|---|---|
| A | III LI8C | 1240 | 104.45 | 55 | 5.90 |
| | III LI9I | 2775 | 105.18 | 50 | 6.40 |
| | Mean | | 104.82 | | 6.15 |
| B | V A29W | 43 | 68.03 | 43 | 3.96 |
| | V A29X | 43 | 66.96 | 43 | 4.79 |
| | V A30S | 43 | 66.29 | 43 | 3.80 |
| | V A30T | 43 | 70.77 | 43 | 5.13 |
| | V A29U | 43 | 68.17 | 43 | 6.46 |
| | V A29V | 43 | 71.89 | 43 | 6.97 |
| | Mean | | 68.69 | | 5.19 |
| | ±S.E. | | ±.89 | | ±.53 |

## DISCUSSION

The acceptance of the purely extracellular origin of the entire fast fraction (or fractions) of the $Na^+$ efflux may at least be attributed to an uncertainty at that time about the volume percentage of the extracellular space in the frog sartorius muscle which varied anywhere from 13% to as high as 40%.[10-18]

The situation has been improved. There are now five independent methods wliicli, in mutual agreement, all yield a value of approximately 9% as the volume percentage of the extracellular space of frog sartorius muscles.[7,8,19,20] A comparison of this 9% figure with the total amount of labeled $Na^+$ in the fast fraction shown in Figure 1. which exceed's 9% by a considerable margin, already shows that the fast fraction could not have originated entirely from the extracellular space. Tlie application of the simple centrifugation method established beyond doubt that the fast fraction is only in part due to tlic extracellular space.

Our next question is, "What is tlie source of the fast fractions of $Na^+$?" Let us examine in light of our present findings some of the answers that have been proposed.

*Zierler's Interpretation of the Fast Fraction:* Zierler and coworkers[21,22] postulated that about 97% of the $Na^+$ in a muscle cell is inside the sarcoplasmic reticulum (SR). Only 1 mmole of $Na^+$ per kilogram of muscle cells is in the "sarcoplasmic space."

It is well known from anatomical studies[23] and from extracellular probe distribution studies that only the T-tubule is open to the exterior and that the space is occupies is negligibly small (0.2 to 0.4%).[24-26] The idea that 97% of the cell $Na^+$ is in the SR involves the postulation that the membrane barrier at the bottom of the T-tubule has pores not permeable to large molecules (e.g., inulin) but freely permeable to $Na^+$. Zierler cited the work of Ling and Kromash[19] which showed that in frog sartorius muscle the sucrose space is larger than inulin space and argued that this membrane is also freely permeable to sucrose.

The data presented in this paper shows that the simultaneous $K^+$ efflux from the same muscle does not show the existence of a corresponding fast fraction. Such a corresponding fast fraction for $K^+$ is mandatory for Zierler's interpretation because (hydrated) $K^+$ is smaller than both (hydrated) $Na^+$ and sucrose.

There is other evidence against Zierler's idea. One example will be mentioned here: In frog ovarian eggs a similar fast fraction of $Na^+$ efflux exists (Ling & Ochsenfeld, 1975, in preparation). Yet frog eggs do not have a SR.

*Conway and Coworkers' Interpretation of the Fast Fraction:* Conway[27] suggested that the fast fraction originates from the sarcolemma. Evidence presented in this paper against the SR interpretation is equally applicable to the sarcolemma interpretation. Indeed it can be cited as evidence against any other similar interpretation of the fast fraction based on an extension of external space.

*Interpretation Based on Two Efflux Mechanisms:* Under equilibrium (or steady-state) conditions, the combination of different modes of permeation will not change the efflux from following a single exponential efflux curve, as long as each of these processes are membrane-limited.[4]

*Heterogeneity in Muscle Cell Population:* A sartorius muscle contains hundreds of muscle cells. Could these cells have different $Na^+$ permeability? This question is well worth raising because Kuffler and Vaughan-Williams[28] discovered that in other types of frog muscles there is a separation into two types of muscle cells. Two reasons, however, rule out this interpretation:

1. Kuffler and Vaughan-Williams found that the muscle cells in the sartorius muscles appear to be uniformly of the fast-fiber type.

2. $Na^+$ efflux from isolated single muscle fibers exhibits similar separation into fast and slow fractions.[2,5]

*Simple Bulk-Phase Limited Diffusion:* Ling, Ochsenfeld and Karreman[29] and Reisin and Ling[30] have shown that in the case of frog ovarian egg and single-isolated giant barnacle muscle cells, the efflux of $H^3H'0$ is best described as bulk-phase limited, with or

without adsorption.

The efflux of $Na^+$ does not follow the kinetics of a simple bulk-phase limited diffusion. If this were the case, then extrapolation of the main portion of the efflux should yield a zero-time intercept equal to about 71% of the total initial $Na^+$ content. In terms of the data given in Table 1, this quantitative relation should predict that the concentration of the slow fraction should be equal to 71% of the total $Na^+$ concentration. The data show that this is not the case.

While simple bulk-phase limited diffusion is ruled out, bulk-phase limited diffusion with adsorption is not. This subject will be discussed in the following section of this paper.

*Sequestration in Subcellular Particle Compartments:* The data presented in this paper are compatible with a model of two (or more) compartments in series. That is, the fast fraction represents membrane permeability-limited exchange and the slow ("30-minute") fraction represents exchange rate-limited by permeability through the membrane of subcellular particles.

A mandatory requirement of this model is that the permeability to $Na^+$ of the subcellular particles must be significantly lower than the cell membrane. Otherwise, a single component efflux will still be the case. Studies of the exchange in isolated mitochondria[31] and nuclei[31] do not suggest that their membranes are less permeable to $Na^+$. In fact, as a rule these membranes are more permeable.

However, it can be argued that this high $Na^+$ permeability of subcellular particles may be due to injury sustained during the isolation process.

A more decisive experimental finding against these two compartment models was derived from the studies of $Na^+$ effluxes in $K^+$-depleted muscles. As is well known, deprivation of $K^+$ increases intracellular $Na^+$. Efflux curves show that this increase represents primarily an increase in the slow fraction (Fraction I). Thus, if the slow fraction truly represents $Na^+$ sequestrated in mitochondria, nuclei, etc., then the whole cell volume would eventually have to be occupied by mitochondria, nuclei. etc., in a muscle whose $K^+$ has been completely replaced by $Na^+$. This prediction is not a reasonable one. It must be pointed out that while this model cannot be the entire answer, on theoretical grounds at least, it cannot be entirely ruled out.

*Two or More Fractions of Intracellular $Na^+$—One Free and the Other Adsorbed:* The existence of intracellular $Na^+$ in two states, one free and the other adsorbed, has been suggested for some time.[33,34] Efflux of labeled $Na^+$ in such theoretical models can produce a complex efflux curve with an initial fast fraction followed by a slower fraction (or fractions) as illustrated in the tandem funnel analogy. In this funnel model, the initial fast flow of water comes largely from the lower funnel with a wide opening to be followed by a slower flow, rate-liniited by the narrower opening of the top funnel.

According to the association-induction hypothesis. $Na^+$ is adsorbed on protein anionic

sites and this desorption may be slow enough that, like the funnel with a narrow opening, it becomes rate-limiting after all the free labeled $Na^+$ in the cell water has exchanged.

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