STUDIES ON ION PERMEABILITY. II. DOES EXCHANGE DIFFUSION MAKE A SIGNIFICANT CONTRIBUTION TO THE NA^+-ION EFFLUX IN FROG MUSCLES?

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SUMMARY

In the preceding paper of this series we showed that in the Na^+-ion efflux curve the slow fraction is rate-limited by desorption from Na^+-ion adsorbing sites and that only the initial fast fraction of the Na^+-ion efflux curve represents the rate of intracellular-extracellular Na^+-ion exchange. We have now shown that the rate of the fast fraction remains the same whether the washing solution contains more than 100 mM of Na^+ ion or whether it contains no Na^+ ion at all. The data indicate that Ussing's "exchange diffusion" does not exist in frog muscle, just as it has been shown to be nonexistent in squid axons, human red blood cells, and mammalian smooth muscle.

In 1943 Levi and Ussing studied the Na^+-ion efflux from frog muscles.[1] The rate of efflux measured was such that an excessive amount of energy would be required to operate the postulated Na pump. To reduce this energy requirement Ussing suggested that a major part of the Na^+-ion efflux from resting frog muscle cells was via a postulated nonenergy-consuming mechanism called "exchange diffusion."[2]

The effectiveness of "exchange diffusion" as a means of reducing energy expenditure relies entirely on its assumption that the "carrier" can travel across the cell membrane only when it is loaded with its passenger Na^+ ion and not otherwise. It is this restriction that would produce a nonenergy-consuming, one-to-one exchange of intracellular and extracellular Na^+ ion.

This fundamental trait of "exchange diffusion" also provides a way in which to evaluate the proportion of Na^+-ion efflux that might be due to the mechanism. If the external solution contains no Na^+ ion at all, the carriers would all be tied up at the outside surface of the cell membrane, and exchange diffusion would come to a halt. Therefore, comparing the Na^+-ion efflux in normal Ringer solution with the efflux in a Na^+-ion-free medium, one could estimate the fraction of Na^+-ion efflux that is mediated by "exchange diffu-

sion." Using this method, Keynes and Swan concluded that 50% of the Na^+-ion efflux from frog muscle is via Ussing's exchange diffusion mechanism.[3]

In the preceding paper of this series, we showed that, contrary to the prediction of the membrane theory, the Na^+-ion efflux from an isolated single frog muscle fiber (as well

imately 30 minutes at 25°C, has been widely accepted as representing the entire intracellular Na^+ ion, its rate being determined by membrane transport. It is now shown to correspond to only a fraction of the intracellular Na^+ ion, the adsorbed fraction, the rate being determined by the rate of desorption.

This finding makes it necessary to re-evaluate many of the Na^+-ion efflux studies published earlier. Keynes and Swan's conclusion that "exchange diffusion" accounts for half of the Na^+-ion efflux from frog muscle can no longer be considered valid, since this conclusion was based on the observation that external Na^+-ion concentration affects the rate of the slow fraction of the Na^+-ion efflux.

The present communication reports the results of studies of the effect of external Na^+-ion concentration on the rate of Na^+-ion exchange during the initial period of a washout experiment. As was shown in the preceding paper, it is this initial fast fraction that reflects the intracellular-extracellular exchange between free intracellular Na^+ and the Na^+ of the external medium.[4] Thus. if exchange diffusion truly exists in frog muscle, the rate of exchange of this fast fraction should depend on the concentration of Na^+ ion in the external medium.

MATERIALS AND METHODS

Isolated sartorius muscles of northern leopard frogs (*Rana pipiens pipiens,* Schreber) were studied, using the U-tube scintillation counter assembly described in the preceding paper.[4] Na^{22} was obtained from Nuclear Science and Engineering, Pittsburgh, Pa. All chemicals were CP grade.

The control washing solution was a Ringer-phosphate containing 104.7 mM NaCl; 2.5 mM KCl; 1.0 mM $CaCl_2$: 1.2 mM $MgSO_4$; 6.1 mM $NaHCO_3$; 2.0 mM NaH_2PO_4; and 1.2 Na_2HPO_4.[5] The various Na-free washing solutions all contained the same concentrations of $CaCl_2$ (1.0 mM) and $MgSO_4$ (1.2 mM) as in the Ringer-phosphate solution. In addition, they contained either of' the following: 117 mM of one of the salts RbCl, KCl, LiCl, choline HCl. NH_4Cl, $(CH_3)_4NHCl$, arginine HCl. or lysine HCl; or 236 mM sugars (sucrose. glucose).

Paired sartorius muscles were tied with bits of colored thread for identification and incubated sterilely overnight at 25°C in a Na^{22}-labeled Ringer-GIB medium capable of

maintaining isolated frog muscles alive for 8 days.[6] Blotted on dampened filter paper according to a procedure described earlier,[7] each muscle was weighed and then lowered into the bottom of a lusteroid counting tube. The radioactivity in the muscles was assayed in a manual well-type 7-scintillation counter (Packard). From the radioactivity counts, the initial labeled Na^+-ion concentration in the tissue was calculated. Previous work has shown that the counting efficiency of Na^{22} is not materially different whether the Na^{22} is inside the muscle cell or whether it is dispersed in 1 ml of 1 N HCl in a lusteroid counting tube.[7]

The control muscle of each pair was then washed in 10 ml of Ringer-phosphate solution in a flask kept in a bath maintained at either 0° or 25° ± 0.05°C and shaken at the rate of 60 excursions per minute. The experimental muscle was similarly washed in one of the Na-free solutions described above. After a specified length of washing time, the radioactivity in the muscles was extracted overnight in 1 ml of 1 N HCl in a glass counting tube and the radioactivity remaining in the tube assayed in a Nuclear Chicago Automatic 7-scintillation counter.

To correct for the differences in counting efficiency in the manual and the automatic counter, the same standard solutions were counted in both counters. From the results, correction factors were derived and applied to the data.

RESULTS

Figure 1 shows the normal Na^+-ion efflux curve from an intact frog sartorius muscle. After correction for the extracellular space (5%, see refs. 7, 8) and connective-tissue contribution (5%, see ref. 4), the curve 'C' was obtained, which could readily be resolved into two fractions (I and II). In the preceding paper of this series we have shown that the slow fraction (Fraction I) is rate-limited by desorption. It is the fast fraction that is rate-limited by the intra-extracellular exchange of free intracellular Na^+-ion. The data also show that after 8 minutes of washing, approximately 50% of the fast fraction (Fraction II) has already exchanged; after 20 minutes of washing, 90%. Data from earlier published work show that roughly the same percentages of exchange of the fast fraction occur in muscles washed at 0°C (ref. 5, p. 328).

Figure 2 shows that the initial Na^+-ion exchange after a two-step washing is not materially different whether the washing solute contains over 100 mM Na^+ ion or whether it contains no Na^+ ion at all.

In most of our studies we exposed the Na^{22}-loaded muscles to a single 20-minute wash at 0°C (Fig. 3). These and other data are summarized in Table 1. There is no significant difference in the percentage of labeled Na^+ ion exchanged between the 47 control muscles washed in a Ringer solution containing about 100 mM Na^+ ion and their pairs washed in 10 different kinds of Na^+-ion-free media ($t = 1.562$ and $p > 0.1$).

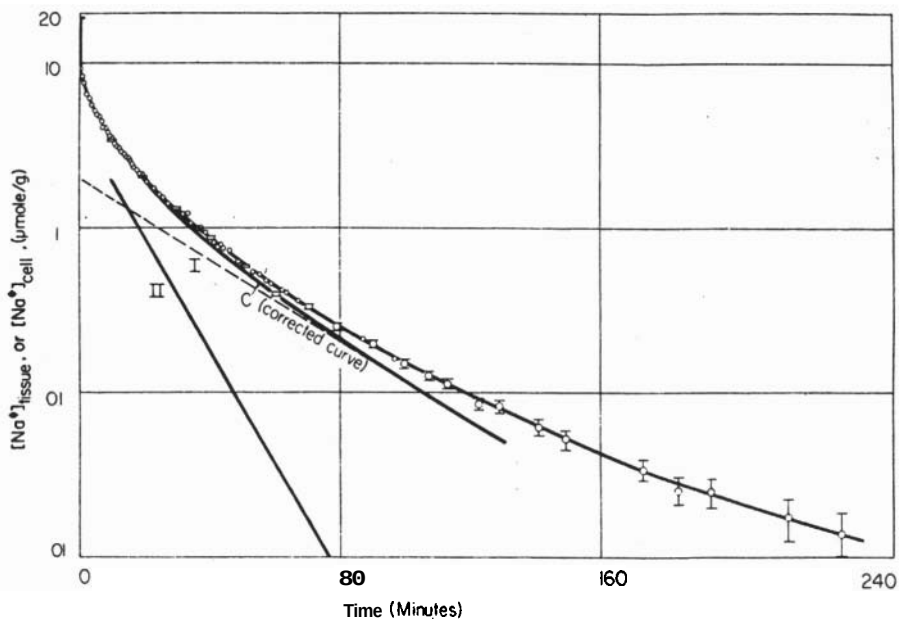

Figure 1. Time course of labeled Na^+-ion efflux from a sartorius muscle washed in normal Ringer-phosphate solution at 25°C.

A sartorius muscle weighing 119 mg was incubated in Ringer-phosphate solution containing Na^{22} for 16 hours at 2°C. Open circles represent the data of labeled Na^+ ion in whole muscle tissue, $[Na^+]_{tissue}$. Correction for the connective tissue was made on the basis of the relation, $[Na^+]_{cell} = 1.1([Na^+]_{tissue} - 0.05\ [Na^+]_{c.t.})$ and a similar efflux curve from a peice of connective tissue isolated from the same leg of the same frog and incubated in the same labeled solution for roughly the same length of time. The connective tissue contains 70% of accessible "extracellular space" water, which is equivalent to .035/.05 or 70% of the extracellular space of the muscle. The remaining 30% of the extracellular space not corrected for in this figure should reduce the initial $[Na^+]_{cell}$ from 19 μmoles/g to not quite 16 μmoles/g. Such a correction would make little change in the part of the corrected curve of interest here. C is the corrected curve which can be resolved into Fractions I and II, Experiment No. 6C16A1.

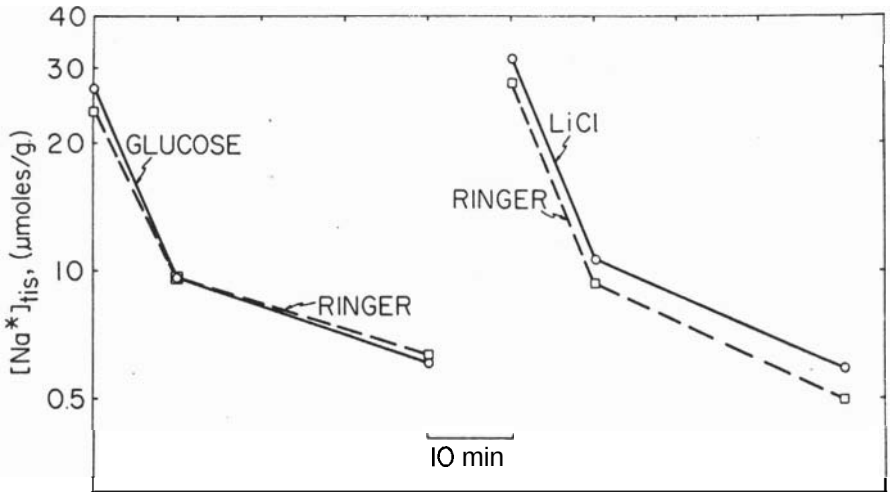

Figure 2. Two-step washout of Na^{22}-labeled Na^+ ion in frog sartorius muscles in normal and Na-free media.
Paired muscles were incubated in Na^{22} containing normal Ringer-phosphate solution at 25°C for 3 h. After blotting, the control muscles were washed for 10 minutes and then for 30 minutes in 10 ml of normal Ringer solution, while their pairs were also washed in two steps in a Na-free glucose or LiCl solution.

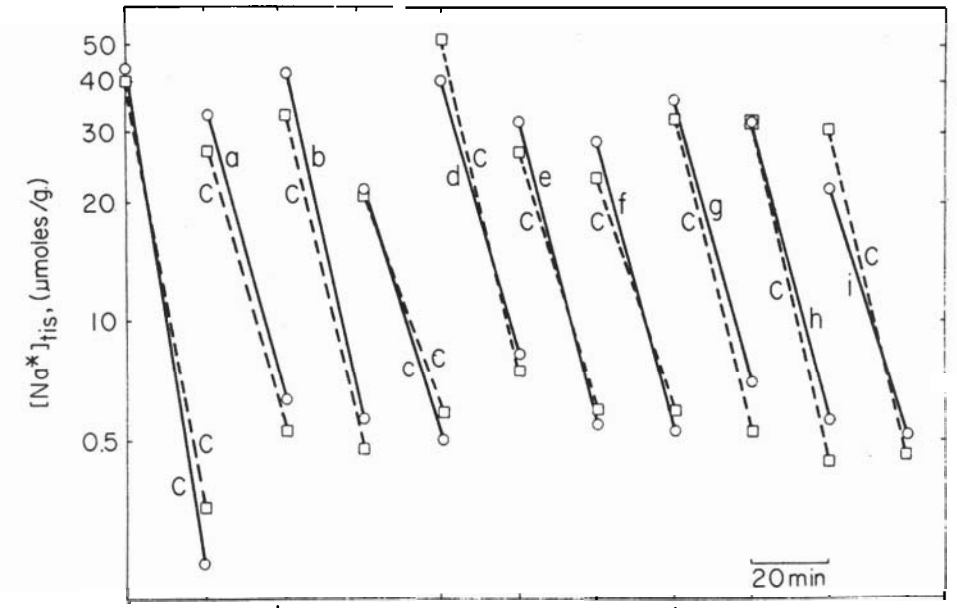

Figure 3. The effect of Na^+-ion deprivation on the initial rate of Na^+-ion efflux from frog sartorius muscle.
Paired sartorius muscles were isolated sterilely and incubated overnight in Na^{22}-containing Ringer-GIB medium at 25°C. After blotting, the control muscles were washed for 20 minutes in 10 ml of cold normal Ringer-phosphate (0°C); the experimental muscles were washed in 10 ml of cold Na-free solution. C indicates control muscles washed in normal Ringer-phosphate; a, glucose; b, LiCl; c, choline chloride: d, $(CH_3)_4NHCl$; e, sucrose; f, RbCl; g, KCl; h, arginine HCl; i, lysine HCl. The data show that the variations between controls and experiments are not greater than the variations between paired control muscles (first pair marked C, C).

Table 1. The Effect of Na^+-Ion Concentration on the Initial Na^+-Ion Efflux of Frog Sartorius Muscles. Values refer to percentages of exchange of Na^{22}-labeled Na^+ ion. NRP refers to normal Ringer-phosphate solution.

	% Na Lost					
Date of Experiment	9-16-69	9-17-69	9-23-69	9-2469	9-25-69	9-31-69
Temperature (C)	25°	25°	0°	0°	0°	0°
Duration of Washing (Min.)	10	8	20	20	20	20
NRP			81.5	89.5	68.7	78.0
NRP			84.0	94.0	71.0	67.0
NRP			68.5		81.2	62.0
NRP			77.0		79.5	65.0
NRP	84.7	77.0	71.3	80.2	81.3	74.0
Glucose	78.8	77.2	81.9	80.9	82.8	78.0
NRP	78.8	77.5	82.8	86.5	78.8	80.0
LiCl	72.3	70.4	83.5	86.7	86.0	73.0
NRP	76.0	79.2	72.5	72.5	79.4	89.0
Choline	73.4	72.9	65.0	76.5	78.0	86.0
NRP		79.0	77.1		94.0	79.0
NH_4Cl		73.8	78.0	67.8	94.4	77.0
NRP		67.7	73.4	85.5	77.4	66.0
$(CH_3)_4NCl$		56.5	74.0	79.5	79.7	65.0
NRP		54.2	76.3	81.7	78.0	84.0
Sucrose		59.5	75.0	83.0	84.5	77.0
NRP				74.4	77.0	73.0
RbCl				81.5	88.4	73.0
NRP			78.0	83.8	79.4	70.0
KCl			71.8	80.5	80.0	66.0
NRP			74.5	86.0	80.1	71.0
Arg. HCl			70.1	82.2	85.0	69.0
NRP			76.5	85.0	69.1	78.0
Lys. HCl			73.5	76.1	77.1	67.0

CONCLUSION

The present investigation shows that there is no evidence that Ussing's "exchange diffusion" mechanism operates in frog muscle at 25°C or at 0°C. This conclusion is in harmony with the earlier conclusion of Hodgkin and Keynes that exchange diffusion does not exist in Loligo and Sepia nerves: with that of Hoffman and Kregenow that it does not exist in human red blood cells,[10] and with that of Buck and Goodford that it does not exist in guinea pig smooth muscle."

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