Physiol. Chem. & Physics 9 (1977)

EXPERIMENTAL VERIFICATION OF AN EXPECTED RELATION BETWEEN TIME OF INCUBATION AND MAGNITUDE OF THE FAST AND SLOW FRACTIONS OF THE SODIUM EFFLUX FROM AMPHIBIAN EGGS

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• ***The Na^+ efflux curves of single ovarian eggs are separable into two fractions. The magnitude of the slow fraction increases slowly with time of exposure of the eggs to labeled Na^+, long after the fast fraction has reached equilibrium. The data agree with the theory that the fast fraction is rate-limited by surface permeation and that the slow fraction is rate-limited by desorption from intracellular adsorption sites.***

INTRODUCTION

In the preceding paper[1] we reported results of studies of the simultaneous effluxes of labeled Na+ and labeled D-arabinose from single frog ovarian eggs. From those results two conclusions were reached: (I) that the slowly exchanging fractions of Na+ (and of D-arabinose) are most likely adsorbed on proteins and possibly other macromolecules, and (2) that the fast fraction represents Na+ (and D-arabinose) in the cell water, its low level reflecting the low solubility of Na+ (and D-arabinose) in the cell water.

A prediction of this model is that by increasing the duration of incubation of the eggs in labeled Na^+, initially both the slow and the fast fraction will increase in magnitude. However, later a point will be reached after which only the magnitude of the slow fraction will increase significantly. Put another way, the ratio of the magnitude of the fast to that of the slow fraction should decline with time. Dick and Lea[2] first pointed out this expectation, which, however, they failed to demonstate.

It was made clear in our preceding paper[1] that while earlier studies unambiguously demonstrated the existence of fractions of Na^+ as well as D-arabinose with different rates of exchange, efflux studies do not always lend themselves to such demonstration. The existence of multiple fractions of Na+ from a single cell with different rates of exchange could be obscured by the slow rate of surface permeation of the labeled solute. Thus we suspect that the failure of Dick and Lea[2] to demonstrate a decreasing ratio of the magnitudes of the fast to slow fraction with increasing duration of exposure to labeled Na+ could simply be a matter of hav-

ing used eggs which happened to have slow surface permeability of Na^+. As the following pages will reveal, in our case the eggs chosen randomly all had fast surface permeation rates and the expected decline of the ratio of the magnitude of the fast to slow fractions was readily confirmed.

MATERIALS AND METHODS

Mature ovarian eggs were isolated from leopard frogs (Rana *pipiens pipiens,* Schreber) *in* late spring. Individual eggs were incubated at 0°C for varying lengths of time in ^{22}Na-labeled Ringer-GIB medium[3] before washing in non-labeled Ringer phosphate solutions. The materials used as well as the basic procedures were essentially the same as those described in full in the preceding paper.'

RESULTS

Figure 1 shows labeled Na+ efflux curves from single frog ovarian eggs which

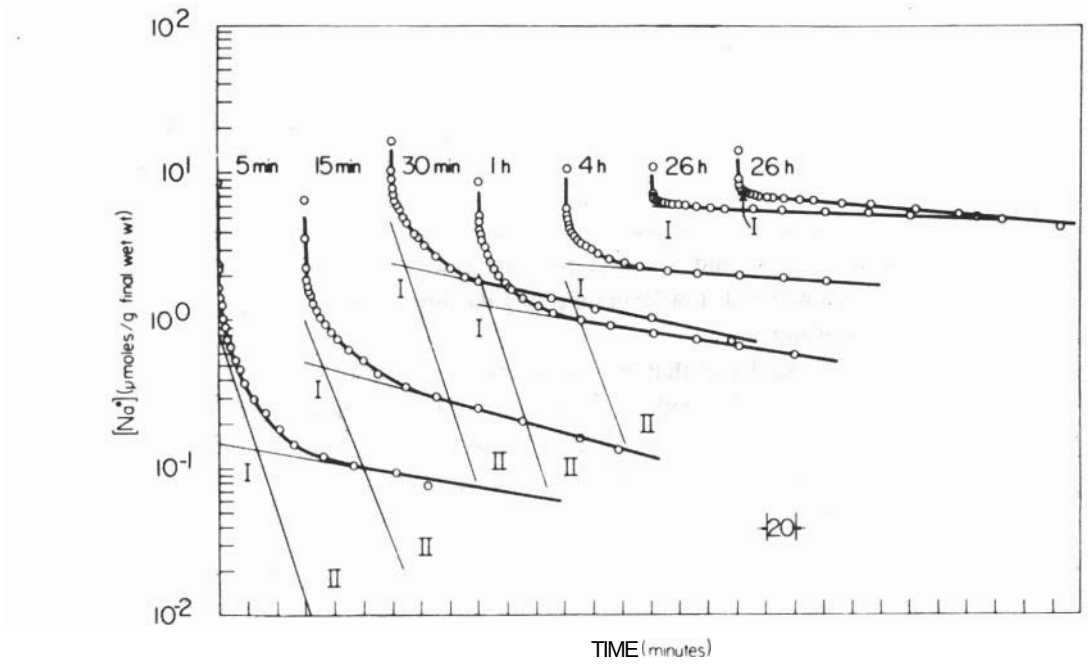

FIGURE 1. Labeled Na' efflux of single frog ovarian eggs after exposure to labeled Ringer solution for different lengths of time. Mature eggs from late spring frogs with a mean water content of **50.3%** and a mean diameter of **1.72** mm were used. Exposure times ranged from 5 **min** to **26** h as indicated in graph. Final net weight of eggs for each exposure period **was 2.42, 2.47, 1.89, 2.28, 2.03, 2.28, 1.88** mg respectively. Distance between ends of arrows represents **20 min** time interval. Lines representing the fast fraction (Fraction 11) are not given for eggs with **26** h of exposure to labeled Na'.

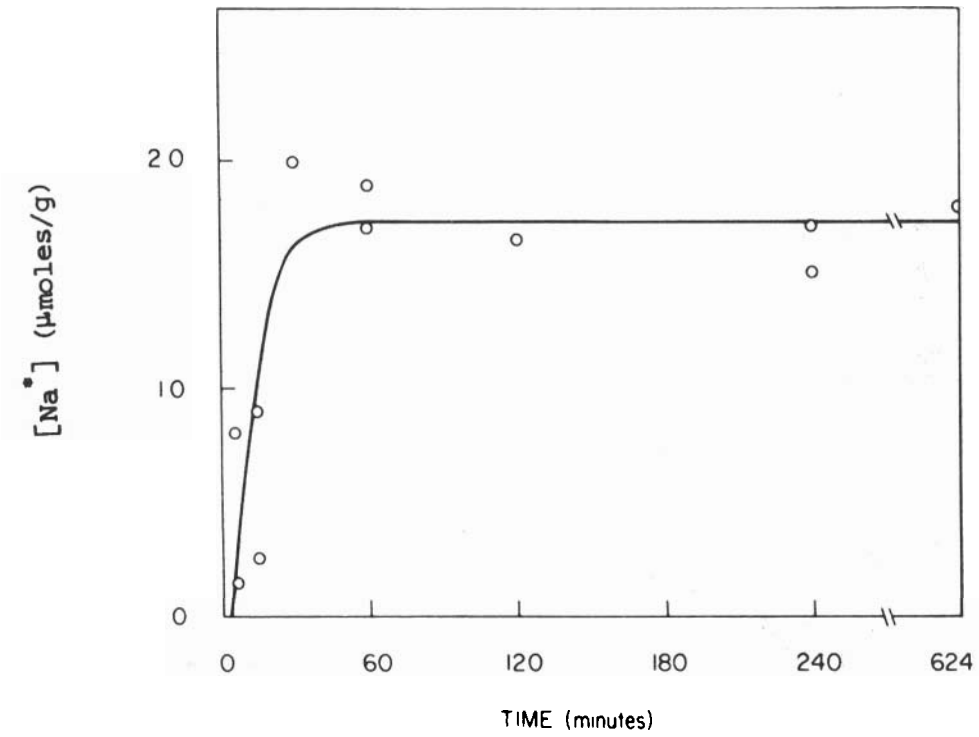

FIGURE 2. Time course of increase in magnitude of the fast fraction of labeled Na^+ with increasing duration of exposure to labeled Na^+. Data indicate rapid increase, with attainment of equilibrium in about 30 min.

had been exposed for different lengths of time to labeled Na^+. The heavier lines going through most of the experimental points have been corrected for contribution from connective tissues which had been similarly exposed to labeled solute for varying lengths of time. By extrapolating from the straight portion of the curves to zero time, one obtains the magnitude of the slow fraction (Fraction **I**). By subtracting Fraction **I** from the corrected curves, one then obtains Fraction **II**, its magnitude obtained from the interception of the straight line with the ordinate. Figures 2 and **3** plot respectively the magnitude of Fractions **II** and **I** against the time of incubation in the labeled solutions. These figures show that whereas Fraction **II** reached equilibrium in approximately **30** min, Fraction **I** continued to increase long after 4 h of incubation. These data agree well with the half time of exchange ($t_{1/2}$) from the efflux curves, which for Fraction **II** was **13.0** ± 0.79 **min** (n = **13**) and for Fraction **I** was 438 ± **73** min (n = **13**). These $t_{1/2}$ values correspond respectively to the time of **90%** exchange of **44** (Fraction **II**) and 1,400 min (Fraction **I**) respectively.

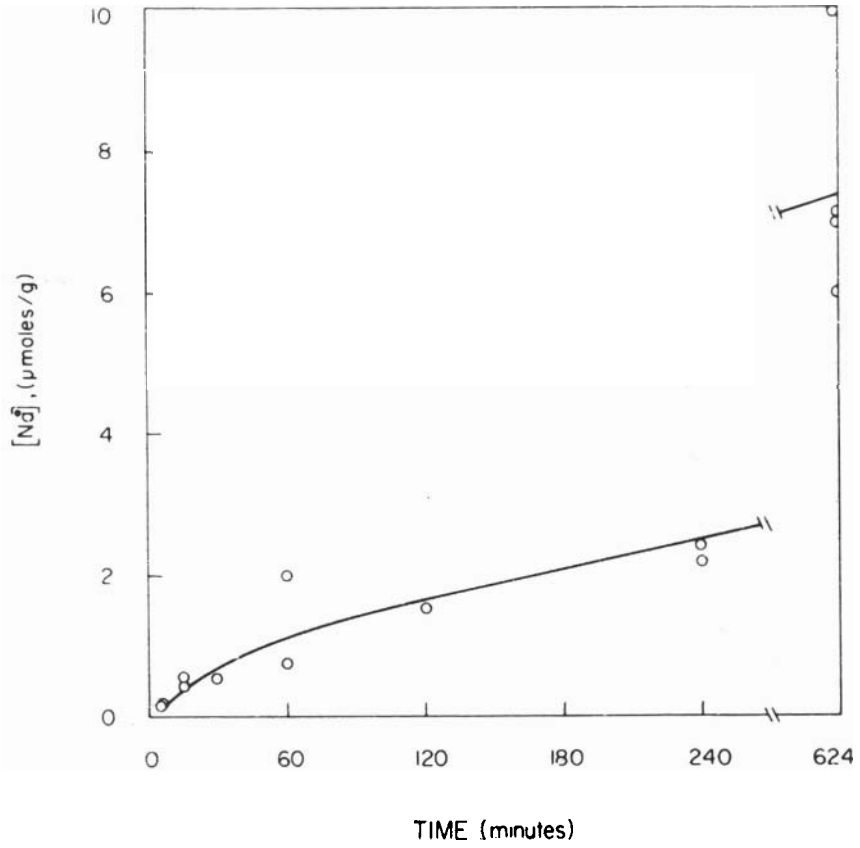

FIGURE 3. Time course of the increase in magnitude of the slow fraction of labeled Na^+ with increasing duration of exposure to labeled Na^+. Data indicate slow gain and failure to attain equilibrium even after exposure of 26 h.

CONCLUSION

The results presented in this paper verify the expectation that by increasing the duration of exposure to labeled Na^+, one increases the ratio of the magnitude of the slow fraction of effluxing Na^+ to that of the fast fraction.

The foregoing work was supported by NIH grants 2-ROI-CA16301-03 and 2-R01-GM11422-13, and Office of Naval Research Contract Number N00014-71-C-0178. The John H. Hartford Foundation provided many of our basic facilities.

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(Received July 27,1977)