THE USE OF ION-SELECTIVE ELECTRODES TO MONITOR FLUXES OF SODIUM AND POTASSIUM IN GLYCERA DIBRANCHATA RBC SUSPENSIONS

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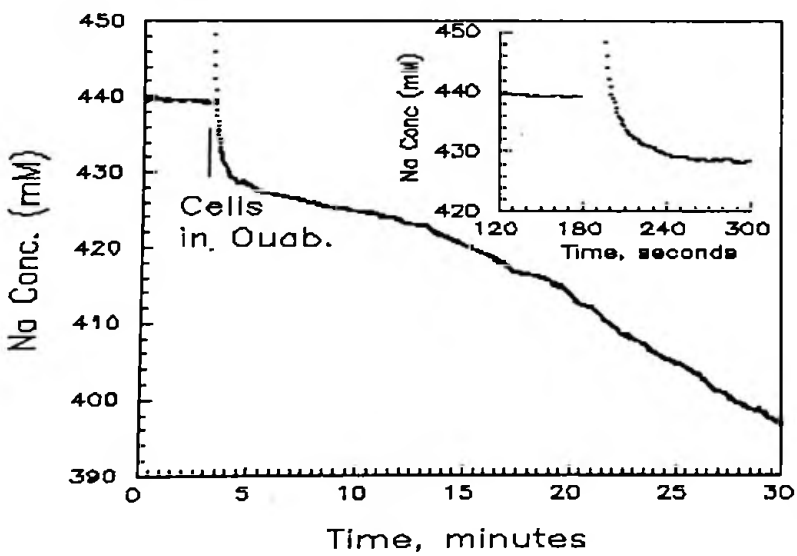
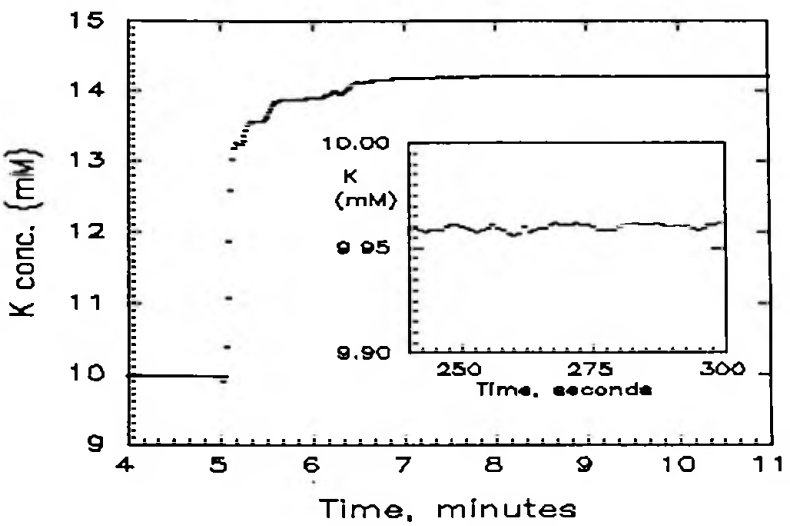
Ion fluxes across the membranes of cells in suspension are conventionally measured by radioisotopes, measuring the changes in nuclide content of the cell and correcting for trapped solution. While accurate, this procedure is tedious, expensive and increasingly subject to regulations. With the development of small ion-selective electrodes, inexpensive high-impedance electronic amplifiers and digital signal processing methods, it is now possible to monitor fluxes by measuring changes in concentration in the extracellular suspending medium. We report on one practical implementation of such a system.

Following the previous report of Brady et al. (Am. J. Physiol. 256:C1105, 1989) we used a Microelectrodes, Inc. (Londondery, NH) Na^+-selective electrode with a WPI (Sarasota, FL) "Ultrawick" reference electrode, connected to a high impedance (10^{15} ohm) amplifier system and an analog-to-digital converter installed in an Apple II computer, using a modulation/data collection system which rejects 60 Hz interference and provides an ultimate resolution of about 100 uV (Kidder, CABIOS 4:331, 1988). This system also performs the voltage/concentration calculations and stores the data to magnetic disk for subsequent analysis, which can include smoothing for noise reduction. The sample cell was carefully thermostatted at 12°C, stirred with a small magnetic stirrer, and electrostatically shielded to further reduce noise. The low-level amplifier stages were battery powered to provide decoupling from AC line transients.



The adjacent figure shows a typical run. Glycera RBC's were equilibrated in artificial sea water (ASW, 440 mM Na). A heavy suspension (50% hematocrit) was prepared in 10 mM ouabane. The run was started at zero time with ASW; the chamber was emptied and refilled with the cell suspension at 3 minutes, at which time the cells had been exposed to ouabain for 10 minutes. The immediate drop in recorded Na concentration indicates that these cells had already removed some Na from the solution during the 10 minute preincubation; the continued and increasing slope of the trace shows increased uptake (increased Na/K pump inhibition) with time. The inset graph shows a magnified view of the period surrounding cell addition, to indicate the time response of the system.



Since the electrode response is Nernstian, the sensitivity in concentration terms is inversely related to the external ion concentration. Since we can easily detect a change of 1 mM Na in ASW, we should detect 0.5 mM in elasmobranch Ringers and 250 uM in mammalian extracellular fluids. This also implies that a similar K electrode operating at 10 mM K should detect changes of a few micromolar K. The figure above shows this apparatus used with a K-selective electrode; the inset shows the sensitivity of these measurements. This figure demonstrates another useful feature of this system. The addition of 0.005% Triton X-100 to the cell suspension at t = 5 min. rapidly lyses the cells, resulting in the release of cellular K into the medium. The relationship between cell water volume, V_c, and total volume in the cuvette, V_t, is given by

$$V_c/V_t = (C_f - C_s) / (C_c - C_s) ,$$

where the subscripts s and f denote the measured starting and final concentrations in the medium, and c is the cellular ion concentration. Thus if we have other information as to C_c, we can determine the effective hematocrit in water volume terms. Alternatively, if we have the hematocrit and the percentage of cell volume which is water, we can rapidly determine cell ion concentrations. In the example shown, C_s is 9.96 mM, C_f is 14.21 mM and the assumed cell concentration is 190 mM; the cell water volume was therefore 2.36% of the total fluid in the cuvette after detergent addition. The inset shows an expanded version of the pre-addition portion of the trace. The total noise and drift in this record is contained within a 10 uM band. It is clear that for many purposes it will be better to monitor K than Na, as for instance in a K/Na exchange system.

The computer system is capable of recording up to 4 electrode channels simultaneously from a sample of 200 ul or less. We intend to construct the necessary apparatus simultaneously to record the solution concentrations of Na, K and H. We believe that this apparatus will be particularly valuable for studies of coupled fluxes in cells and vesicles, since in many systems in which an organic molecule is co- or counter-transported with an ion, the flux of the organic moiety has been measured as a function of the ion concentration but not vice-versa. This apparatus should make such measurements feasible.

We thank Dr. Rolf Kinne for supplying the glass cuvettes used in the later parts of this study. Supported in part by Illinois State Univ. (to GWK) and National Sea Grant and NIEHS grant ES0382-06 (to RLP).