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Using a Calcium Electrode to Measure Mercury in Aqueous Solutions

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The development of small specific-ion electrodes and computer signal processing has made it possible to measure the uptake or release of ions from concentrated cell suspensions by monitoring the changes in activity in the suspending solution (Kidder and Preston 1993). In preparation for studies of the effect of mercury on ion transport across cell membranes, the effect of $HgCl_2$ on the electrodes' output was measured. A rapid and reversible depression of Ca-electrode voltage by mercury was seen, which can be used to measure mercury concentrations under suitable conditions. Several examples are shown which support a diffusion/binding model for cellular uptake of extracellular mercury. Since there are no electrodes which are specifically sensitive to mercury, this method may be important in studies of mercury toxicity and for environmental monitoring purposes.

MATERIALS AND METHODS

A block diagram of the apparatus is shown in Figure 1. The Ca electrode (Microelectrodes, Inc. Model MI-600) has a resin membrane with excellent selectivity specifications; Ca^{2+}/Mg^{2+} = 20,000, Ca^{2+}/Na^{+} = 10,000, Ca^{2+}/K^{+} = 10,000. The K electrode is also a resin variety, while the Na and H electrodes have glass membranes. The cation selective electrodes were operated

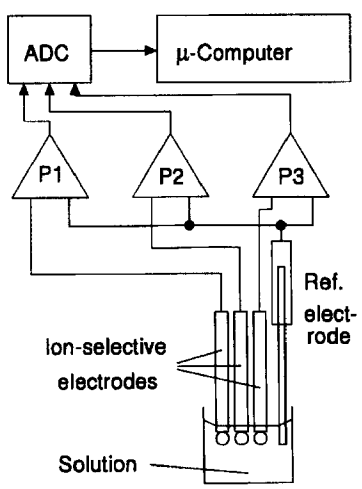

Figure 1. Block diagram of the apparatus. Three electrodes plus reference are illustrated, in a sample volume of 300-500 μl. Preamplifiers (P1-P3) are Analog Devices AD549 and drive the computer data system, which samples each channel once per second for a maximum of 20 minutes.

with a common reference electrode (WPI, Inc. Model MERE-1). An Apple IIe microcomputer with an 8-bit analog-to-digital converter (ADC) was used for data collection and analysis; a modulation system (Kidder 1988) increased the recorded resolution to about 10 bits, which with amplification resulted in a resolution of about 100 μV, below the noise level of these electrodes. The system was thermostatted to 18° for these experiments on cold-water marine species.

Table 1. Composition of solutions.

Substance	Artificial Sea Water (mM)	Elasmobranch Ringers (mM)
NaCl	440	268
KCl	9	6
$CaCl_2$	9.3	5
$MgCl_2$	23	3
$KHCO_3$	2.2	-
$NaHCO_3$	-	20
Urea	-	350
Glucose	-	5

Two physiological salt solutions were used for these experiments. When coelomocytes (red blood cells) from *Glycera dibranchata* (the marine bloodworm) were used, the suspending solution was artificial sea water (ASW). Red blood cells from *Raja erinacea* (the little skate) and rectal gland cells from *Squalus acanthias* (the spiny dogfish) required elasmobranch Ringers solution (ER). The compositions of these solutions are given in Table 1. Electrode calibration was routinely performed by adding a small volume of a concentrated solution (mM: NaCl, 1466; KCl, 30; $CaCl_2$, 31) or H_2O to achieve small concentration increases or decreases.

A stock solution of 100 mM $HgCl_2$ in water was prepared. When millimolar concentrations were needed this could be added directly, with proper compensation for the small dilution of the solution electrolytes. For lower concentrations this stock solution was diluted in the appropriate physiological solution before addition.

Glycera dibranchata was collected by commercial harvesters from mud flats on the borders of the Gulf of Maine, and maintained in running sea water until used. The coelomocytes were collected (Chen and Preston 1987) by expressing the coelomic cavity contents into ASW; repeated centrifugation and resuspension was used to separate the coelomycetes from gametes which were often present in quantity. *Raja erinacea* were collected by ground trolling in the Gulf of Maine and maintained in running sea water until needed. Blood was collected from the caudal vein with a heparinized syringe and needle, and washed by centrifugation in ER. When blood was stored overnight at 4° for use the following day, further centrifugation was performed to rid the solution of hemolysis products. Rectal gland cells from *Squalus acanthias* were the kind gift of Dr. Richard Solomon.

All calibrations and calculations were performed on the assumption of complete ionization, as if the activity coefficients for all species were unity. Over this limited range of major ion concentrations, the errors from violation of this assumption will be negligible for the present purposes. Mercury in solution exists in a variety of

forms (see Discussion); for our purposes, the abbreviation "Hg" will indicate total mercury.

RESULTS AND DISCUSSION

Initially, the Ca electrode was calibrated across a small range of Ca^{2+} concentrations around that present in the suspending solution, for use in studies of the influence of Hg on Ca^{2+} flux. The effect of added $HgCl_2$ was tested, to see if electrode measurements would be valid. Six calibration runs were performed, alternating between control and 20 μM $HgCl_2$ in ASW. The individual records show a consistent depression of electrode voltage upon the addition of this concentration of $HgCl_2$. When the data are plotted as in Figure 2, it is clear that the effect of Hg is on the offset voltage, not on the slope, but that the offset does not reach statistical significance with this number of points. It would appear that the calcium electrode can be used to measure Ca^{2+} in the presence of a constant concentration of Hg in this range, if appropriately calibrated.

When the calcium concentration is fixed at its normal solution value (see Table 1) and $HgCl_2$ is added over a wider range, the electrode can be calibrated to measure mercury concentration. Figure 3 shows a calibration curve in artificial sea water, while Figure 4 shows the response of the Ca electrode to changes in Hg concentration in elasmobranch Ringers. The calibration curve retains the same form in the two solutions, but the coefficients are markedly different.

When the cuvette contains a suspension of dogfish rectal gland cells, the

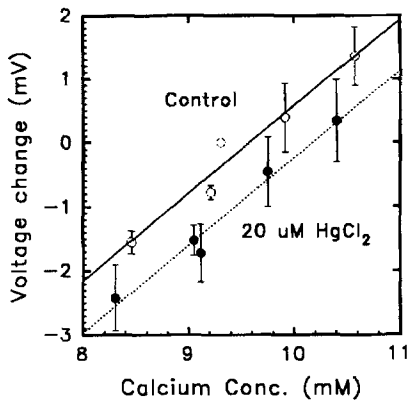

Figure 2. Calibration of the Ca electrode with (solid points) and without (open points) 20 μM $HgCl_2$. Mean $\pm$ SE for 3 observations at each concentration; lines linear least-squares fit.

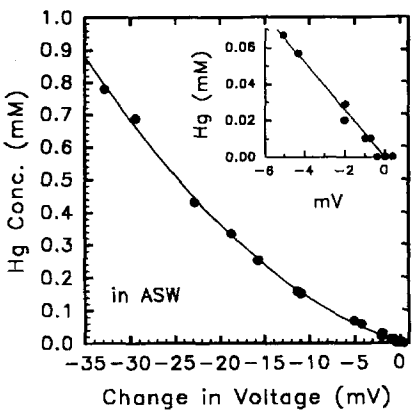

Figure 3. Voltage change of Ca electrode vs. $HgCl_2$ concentration in ASW (Ca concentration 9.3 mM). Data best fit by second-order equation $Hg = a + bV + cV^2$ (μM, mV, $\pm$ SD) where $a = 2.603 \pm 3.664$, $b = -8.829 \pm 0.785$ and $c = 0.437 \pm 0.026$. At low concentrations (inset) the linear approximation $Hg = a + bV$ is sufficient, for which $a = 0.000 \pm 0.113$ and $b = -7.620 \pm 0.371$.

addition of $HgCl_2$ results in a characteristic response of the Ca electrode voltage, as seen in Figure 5. The immediate voltage response to the addition of mercury is largely restricted to the calcium electrode, which is also seen in calibration runs. There is no detectable response from the Na electrode and only a small negative-going "step" response from the K electrode, followed by a slow change which probably indicates K^+ loss from the cells. In this poorly buffered solution, it is probable that the response of the H^+ electrode is due to a real pH decrease. The Ca electrode shows an immediate negative-going voltage spike followed by a return toward its previous voltage. This response is characteristic of the addition of $HgCl_2$ to a suspension of all cell types tested.

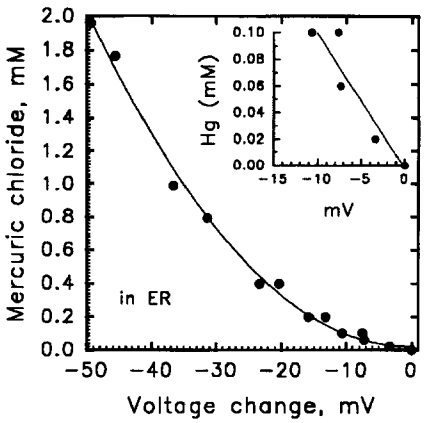

Figure 4. Voltage change as a function of Hg concentration in elasmobranch Ringers (Ca concentration 5 mM). Second-order fit; a = 0.022 ± 0.008, b = 0.0014 ± 0.0009, c = 0.000837 ± 0.0000183. Inset; a = -0.00228 ± 0.00495, b = -0.0102 ± 0.0008.

Using the calibration curve established for this solution (Figure 4), the change in Ca electrode voltage can be converted to the equivalent mercury concentration, and plotted as shown in Figure 6. The Hg concentration "spikes" and then returns toward zero along a quasi-exponential decay curve. A reasonable explanation for this curve is that the added Hg is being removed from the solution by simple diffusion across the plasma membrane into the cell cytoplasm, where an avid binding mechanism keeps the intracellular concentration of "free" Hg very low. If this is so, the slope of the exponential uptake should be proportional to the cell number but independent of the initial mercury concentration, while the intercept should be proportional to the mercury concentration but independent of cell number. Using coelomycetes from *Glycera* as a convenient source of high cell concentrations, we can test these predictions. Figure 7 shows the addition of the same amount of mercury to cell suspensions of two different

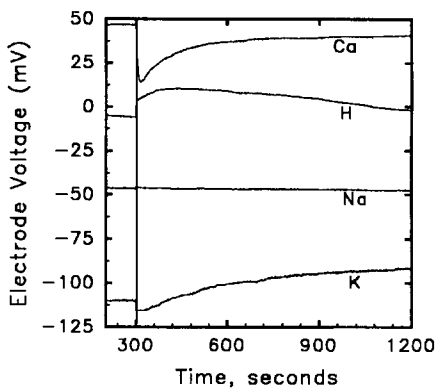

Figure 5. Effect of $HgCl_2$ on voltage from 4 electrodes in a dilute suspension of rectal gland cells from dogfish. At 300 sec, 5 μl of 100 mM $HgCl_2$ was added. The Ca electrode responds with a rapid "spike" followed by a return toward its initial voltage.

densities, while Figure 8 shows two different mercury concentrations added to suspensions of the same density. Finally, the cells were lysed with the nonionic detergent Triton X-100 and the mercury addition repeated on the lysate, with the results shown as Figure 9. With the cell membrane disrupted, there is no electrode response to added $HgCl_2$.

Aqueous solutions of mercuric chloride contain many mercury species in equilibrium, including Hg^{2+}, $HgCl^+$, $HgCl_2$, HgOHCl, $HgCl_3^-$ and $HgCl_4^{2-}$; the latter is the majority species (80%) in sea water (Webb 1966). Gutknecht (1981) has concluded that $HgCl_2$ is the only species which crosses artificial lipid bilayers at a significant rate, and that permeability of his membranes to this species is very high (1.3×10^{-2} cm·sec^{-1}). Others (Passow and Rothstein 1960, Rothstein 1973) have reached similar conclusions for cell membranes. In the present experiments, total Hg was used to calculate the calibration curves, since we do not know which species interacts with the Ca electrode. Since all Hg species are in rapid equilibrium, and since the conditions which could shift that equilibrium (e.g., Cl^- concentration) are constant during any experiment, the proportions of these species will be constant. The electrode offers the possibility of resolving some of these questions, but this is outside the scope of this contribution. I use the abbreviation "Hg" to indicate total mercury, being that mix of mercury species resulting from the addition of $HgCl_2$ to the biological solution.

Several things should be noted about the mercury calibration curves shown in

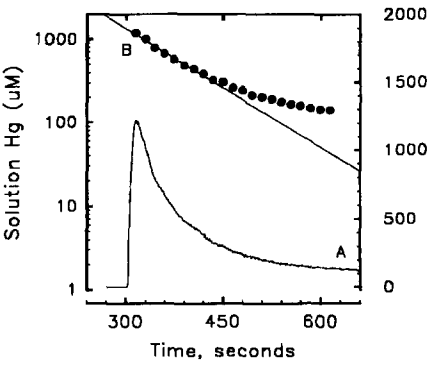

Figure 6. A portion of the Ca electrode response from Figure 5, converted to concentrations using the calibration curve from Figure 4. Curve A is plotted on a linear scale (right axis); curve B shows these same data in log transformation, with a straight line fit through an 80 second portion following the voltage spike.

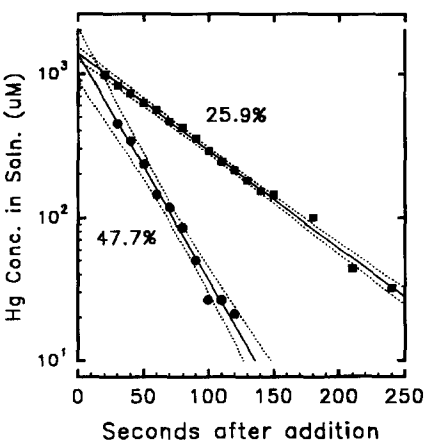

Figure 7. Hg disappearance curves for two hematocrits of *Glycera* RBCs. In separate experiments, 5 μl of 100 mM $HgCl_2$ was added to 500 μl of a suspension of RBCs in ASW. The Hg concentration was plotted, using the post-transient part of the response. Solid lines are the best linear fit; dotted lines the 99% confidence limits.

Figures 2 and 3. First, the voltage depression upon the addition of Hg is rapid and reversible, with a time constant (1 to 5 sec) about equal to that observed for changes in Ca^{2+} concentration. Second, the voltage response is not that predicted by the Nernst equation, which suggests that mercury inhibits the response of the electrode to Ca^{2+}, but is not substituting for calcium. Third, the response is well fit by a second degree polynomial of the form $[Hg] = a + bV + cV^2$, where $a \approx 0$. A stable calibration curve such as this is just as reliable as a straight line relationship, although it requires more complex calculations. For low [Hg] a linear approximation is sufficient. It is important that the electrode be calibrated in the solution in which it is to be used.

In cell suspensions, Hg disappears from the extracellular solution with an exponential time course, with essentially all of the mercury being removed. This is easily explained if the free mercury concentration within the cells remains essentially zero, which requires Hg binding sites of sufficient avidity and capacity to accommodate the entire amount of mercury added. Even when a large initial concentration of Hg (1 mM) is used with a low cell volume (20% hematocrit), the concentration of binding sites within the cell need only be equivalent to 5 mM if the association coefficient is large. On the assumption that compounds such as sulfhydryl-containing amino acids (free or in proteins) might be a major binding site, such a concentration is not unreasonable. Other organic ligands such as histidine are probably also involved (Webb 1966). Since lysis of the cells does not release Hg in a form

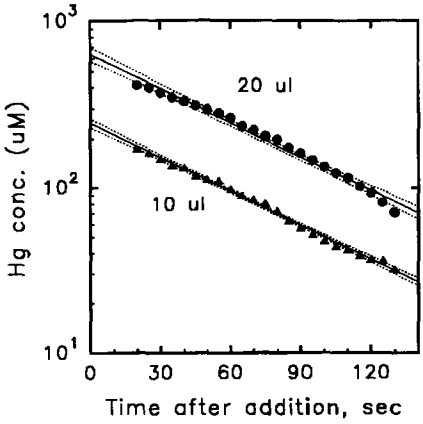

Figure 8. Mercury disappearance curves for two concentrations of Hg using *Glycera* cells at the same hematocrit (20.1%). In separate experiments, 10 and 20 μl of 10 mM $HgCl_2$ was added to 300 μl of cell suspension. Meaning of lines as in Figure 7.

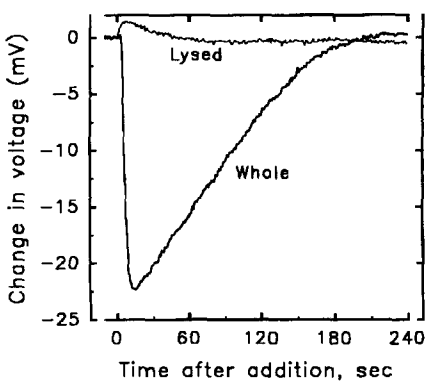

Figure 9. Ca electrode voltage change following the addition of Hg to a suspension of *Glycera* RBCs, before and after lysis by 0.3% Triton X-100. The Hg concentration in the absence of cells would have been 625 μM. The characteristic spike upon Hg addition is abolished by hemolysis and release of the cytoplasmic contents into the bathing medium.

measurable by the electrode, and since the cell lysate apparently binds Hg very rapidly (Figure 9), the finite time course for Hg disappearance from the medium in the presence of cells can be explained by diffusion of Hg across the cell membrane and subsequent binding in the cytoplasm.

This situation mimics the conditions used by Gutknecht (1981) who added 1 mM EDTA to the *trans* solution to keep the concentration of inorganic Hg close to zero, thus allowing equation of net flux and one-way (*cis* to *trans*) flux. Under these conditions, the Hg concentration in the *cis* or extracellular solution should decrease along a single exponential with a time constant proportional to the permeability coefficient of the membrane times the membrane area. In the present experiments, a short time is required following the addition for Hg to distribute uniformly through the extracellular fluid and for the electrode to react to Hg, accounting for the rising phase of the concentration-time curve. After this transient has dissipated (5-10 seconds), the plot of [Hg] vs time should be exponential. In the presence of higher initial Hg concentrations and/or lower cell densities, it is frequently found that the subsequent "exponential" can be concave upward (e.g., Figure 6) or downward (e.g., Figure 8). The former would be predicted if the amount of mercury added exceeded the binding capacity of the cells, resulting in a finite free Hg concentration in the cytoplasm (and therefore in the extracellular fluid) at equilibrium. The concave-downward "exponential" would occur if some mechanism other than simple diffusion influenced the rate at which mercury can cross the membrane. Any saturable process, such as carrier mediated diffusion or single file diffusion, would produce this result, as would multiple exponentials due to the existence of several compartments in series, which could be spaces within nuclei, mitochondria, etc. These data are not sufficient to discriminate between such theoretical options. Departures from exponential behavior will change the concentration determined by extrapolation of the line to the moment of addition, and may explain the observation that while these extrapolated values agree closely with the values calculated from the amount of mercury added to the extracellular water at low mercury concentrations, there can be significant errors at higher mercury concentrations.

The calcium-selective electrode can thus be used to measure mercury concentrations in biological solutions, and gives rapid and sensitive measurements between 10 μM and above 2 mM when appropriately calibrated. Since the calibration curve is sensitive to calcium concentration, it is necessary that Ca^{2+} in the solution be known and constant. These electrodes can be used for their original purpose in solutions containing constant mercury concentration if the resulting offset voltage is corrected. As calibrated, this method measures total inorganic mercury, and further experiments will be necessary to determine the species to which the electrode reacts. This method can provide a rapid measure of mercury concentration changes, which should be useful for studies of the interaction of this important toxin with biological systems.

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REFERENCES

Chen CW, Preston RL (1987) Effect of mercury on taurine transport by the red blood cells of the marine polychaete, *Glycera dibranchiata*. Bull Environ Contam Toxicol 39:202-208

Gutknecht J (1981) Inorganic mercury (Hg^{2+}) transport through lipid bilayer membranes. J Memb Biol 61:61-66

Kidder GW III, Preston RL (1993) The use of ion-selective electrodes to monitor fluxes of sodium and potassium in *Glycera dibranchata* RBC suspensions. Bull Mt Desert Isl Biol Lab 32:30-31.

Kidder GW III (1988) Resolution improvement by modulation of analog-to-digital converters. Comp Appl Biol Sci 4:331-335

Passow H, Rothstein A (1960) The binding of mercury to the yeast cell in relation to changes in permeability. J Gen Physiol 43:621-633

Rothstein A (1973) Mercaptans, the biological targets for mercurials. In: Miller, MW and Clarkson TW (Eds) *Mercury, Mercurials, and Mercaptans* Charles Thomas, Springfield, Illinois. pp 68-72

Webb JL (1966) *Enzyme and Metabolic Inhibitors* Vol 2, Ch 7 Mercurials. Academic Press New York pp 729-9986