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Electrochemical performance of an ion selective, polymeric membrane following chronic implantation in rat subcutaneous tissue

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Abstract

To determine if ion selective, poly (vinyl chloride) (PVC) membranes retain their electrochemical properties following chronic exposure to a physiologic environment, miniaturized, H^+-selective electrodes are implanted subcutaneously in the rat for up to 21 days. Electrode stability, sensitivity, selectivity, response time and internal resistance are measured following implantation and compared to pre-implant values. We show that sensitivity and selectivity are decreased only slightly following implantation. Electrode stability and response times show small changes over implant duration. We conclude that, with the exception of drift, ion selective, PVC membranes retain much of their capacity for accurate electrochemical measures following as much as 21 days of direct and continuous exposure to rat subcutaneous tissue.

Keywords: Ion selective electrode; Polymer membrane; Rat; Subcutaneous; In vivo

1. Introduction

Liquid membrane ion selective electrodes are widely used to measure ion activity in blood samples using bench-top chemical analyzers [1], and may be used to continuously measure ions in blood for periods of hours [2]. However, longer or repeated exposure to biological fluids can cause degradation in electrochemical performance [3] and, even with the use of biocompatible coatings [4], it is not clear that electroactive membranes can be used for accurate in vivo ion determination over periods of days and weeks. NASA researchers need to continuously monitor chemical signals in space flight animal models for periods of weeks to months [5]. Pediatric surgeons at the University of California, San Francisco need to continuously monitor the human fetus in utero following open fetal surgery [6]. NASA and UCSF are collaborating on the development of chemical sensors, animal models, and wireless signal acquisition devices for the purpose of evaluating and improving the chronic performance of ion selective membranes in vivo.

To determine if ion selective, poly (vinyl chloride) (PVC) membranes retain their electrochemical properties following chronic exposure to a physiologic environment, miniaturized, H^+ selective electrodes were implanted subcutaneously in the rat for up to 21 days. These electrodes incorporated a plasticized, PVC membrane doped with an H^+-selective, neutral carrier ionophore. We also implanted miniaturized reference electrodes incorporating a poly (hydroxyethylmethacrylate) (p-HEMA) frit. Electrode performance characteristics including stability, sensitivity, selectivity, response time, and internal resistance were measured and compared before and after implantation.

2. Experimental

2.1. Chemicals

Double-distilled water and analytical reagent-grade chemicals were used in all experiments. Membrane solutions were prepared according to the recipe of Lemke et al. [7], and included high molecular weight PVC, bis (2-ethylhexyl) adipate as a plasticizer, potassium tetrakis (4-chlorophenyl) borate as a lipophylic salt, and tridodecy-

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lamine as the proton ionophore. Tetrahydrofuran was used as the solvent. Reference electrode liquid junctions were fabricated with a polymerized HEMA monomer solution described by Koudelka-Hep et al. [8], containing hydroxyethyl methacrylate (HEMA), dimethoxyphenylacetaphenone, polyvinylpyrrolidone, tetraethyleneglycol dimethacrylate, and ethylene glycol as the solvent.

Phosphate buffered saline (100 mM NaCl, 20 mM Na_2HPO_4, and 5 mM KH_2PO_4) was used as both a storage and test solution. Buffer pH was adjusted with 1 M HCl or 1 M NaOH, and measured with an H^+-selective combination glass electrode (Orion 81-02).

2.2. Electrodes

Both indicator and reference electrodes were fabricated from 3.8 cm sections of a flexible, non-toxic, medical grade PVC tubing, with an outside diameter of 1.7 mm and an inside diameter of 1 mm (Fig. 1). Internal filling solutions consisted of 50–100 mg agar, used as a hydrogel, dissolved in 3 ml of AgCl-saturated, phosphate buffered saline at pH 7.4. Ag/AgCl wire (0.1 mm in diameter) was used as the internal electrode for both indicator and reference half cells. The back end of the electrodes was sealed with an epoxy plug. To make an indicator electrode, the open end of the tubing was dipped in the PVC membrane solution 6 times (10–15 min between dips) and allowed to air dry overnight. To make a reference electrode, the open end of the tubing was filled with 1–2 mm of the HEMA solution which was polymerized with 10–15 min of ultraviolet radiation exposure. Reference electrodes were a modified version of those described by Margules et al.[9].

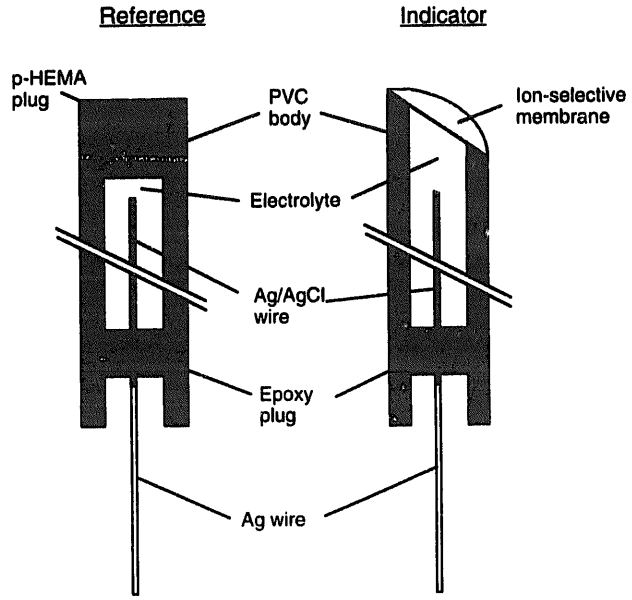

Fig. 1. Schematic diagram of ion selective and reference electrodes.

2.3. Electrochemical measurements

Prior to bench testing, all electrodes were conditioned for approximately 24 h in aqueous buffer solutions at pH 7.4. Electrode potentials were measured at room temperature (19–23°C) with a high impedance pH meter (Orion). Electrochemical measurements included stability, sensitivity, selectivity, and response time. Internal resistances were also measured.

2.3.1. Stability

Electrode stability was quantified by calculating the long-term drift against a commercial single-junction Ag/AgCl reference electrode (Corning). Potentials were measured at the beginning and end of a test period, and the difference in potential was divided by the test duration. Initial tests of 1–6 days in duration were conducted in buffer solution at room temperature, using a computer-controlled, 24-channel switch box (Carsten Mundt, North Carolina State University) to acquire and store cell potentials automatically. Subsequent tests of 4–21 days duration were conducted in buffer solutions at 35°C or in animals, and long-term drift was calculated from cell potentials measured in buffers at room temperature before and after the test.

2.3.2. Sensitivity

The sensitivity of the indicator electrode to pH change was calculated by dividing the difference in potential, determined in conjunction with a commercial reference, by the difference in pH for buffers between pH 6 and 8.

2.3.3. Selectivity

The selectivity of our indicator electrodes against Na^+ was determined using a mixed-solution technique [10]. Cell potential versus pH was measured in a phosphate buffer with 140 mM Na^+ by titration with 1 N HCl. Potentials were adjusted between pH 12 and pH 2, and curves plotted for pH versus emf potential. Selectivity coefficients were calculated according to

$$K_{i,j}^{pot} = (a_i)/(a_j)$$

where (a_i) is the activity of the H^+ ion and (a_j) is the activity (concentration) of the interfering ion Na^+ at the intersection of the two linear portions of the curve in the region of low H^+ activity.

2.3.4. Response time

Response times were determined by moving electrodes between buffers differing in H^+ activities. Response times were defined as the time between the initial deflection from baseline, established at pH 8.0, and the point when the response reached 95% of its final value at pH 7.0. The pH 7.0 buffer was continuously stirred to insure rapid mixing when electrodes were placed in this buffer.

2.3.5. Internal resistance

The internal resistance of the indicator electrode was determined by the voltage divider method using known shunts [11] and is given by

$$R_i = \Delta E^*(R_s/E - \Delta E)$$

where R_s is the shunt resistance, E is the cell potential, and ΔE is the amount the cell potential is reduced with the shunt. The pH of the test electrolyte was adjusted to generate a cell potential between 150 and 200 mV, and the shunt resistance was chosen such that the cell potential was reduced between 10 and 20%. Ag/AgCl wire was used to complete the electrical circuit.

2.4. Animals

2.4.1. Protocol

To determine the effect of subcutaneous implantation in the rat on electrode performance, electrochemical characteristics were measured in standard buffer solutions at room temperature before and after implantation times of 4, 8, 12, and 21 days. Seven experiments were conducted, two each at 4, 8, and 12 days, and one at 21 days. Each experiment used 3 rats with 4 implanted electrodes (2 reference and 2 indicator) in each. For controls, indicator and reference electrodes were placed in a 35°C buffer solution to approximate rat subcutaneous temperature.

2.4.2. Surgery

Twenty-one rats were pre-anesthetized with methoxyflurane (Metafane™) inhalation followed by intramuscular injection of ketamine hydrochloride (60–100 mg/kg, i.m.). Electrodes were inserted into subcutaneous pockets on the backside of each rat such that the H^+ sensitive membrane and reference frit were located at the back of the pocket away from the incision site. The pockets were sutured closed around the electrode resulting in either a transcutaneous or totally implanted electrode configuration. Following surgeries, rats were provided food and water ad libitum. We attempted to acquire cell potentials from transcutaneous electrodes, but difficulties with restraint jackets and tethering conductors precluded consistent data acquisition in vivo. At the selected times, 4, 8, 12, and 21 days post implantation, electrodes were removed from the rats to standard aqueous buffer solutions for testing.

The experiments described in this study were approved by the Institutional Animal Care and Use Committee, and the treatment of animals was in accordance with NIH guidelines.

3. Results

3.1. Preimplant

Of the 92 indicator and 88 reference electrodes fabricated for this study, nearly all exhibited expected electrochemical characteristics in standard buffer solutions. A small number (5 indicator and 6 reference electrodes) failed before or during initial bench tests; in other words exhibited continuous or intermittent electrical shorting or opens, or exhibited large and rapid potential drift. Since selectivity, response time, and internal resistance were not measured in some experiments, these measurements are not reported for all fabricated electrodes.

3.1.1. Stability

At room temperature, both H^+-selective and reference electrodes were stable in phosphate buffered saline solutions (e.g. Fig. 2). Mean drift values centered on zero in both cases, however, ion selective electrodes showed greater variance in drift than reference electrodes (Fig. 3a,b). For test periods between 20 and 140 h conducted prior to implantation, 70% of the indicator electrodes and 95% of reference electrodes drifted less than 2 mV day^{-1}. Since indicator and reference electrodes were constructed identically except for the presence of the PVC membrane, differences in drift are most likely related to the membrane. These drift rates are similar to those reported by others for miniaturized indicator and reference electrodes [9,12].

3.1.2. Sensitivity and selectivity

Indicator electrodes showed approximately Nernstian sensitivities (mean slope = 57.1 mV/pH; SD 0.9, $n = 89$ electrodes) and high selectivities against Na^+ (mean log $K_{i,j}^{pot} = -9.8$, SD 0.2, $n = 67$ electrodes) at room temperatures (19–23°C) (Fig. 3c,d). The range of sensitivities we observed, 55–59 mV/pH, was similar to that reported previously for the same membrane cocktail [7]. Similar values of selectivity coefficients have been reported for the tridodecylamine ionophore [13].

3.1.3. Response times and internal resistance

The majority of our indicator electrodes showed fast response times and relatively low resistances. Nearly 90% of the sensors responded within 2.5 s for step changes in

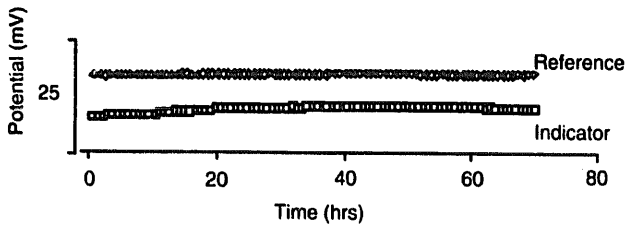

Fig. 2. Indicator and reference electrode stability. Raw emf measurements were taken over a 70 h period. Potentials were measured every 24 min from each ISE and reference electrode in combination with a commercial Ag/AgCl single-junction reference (Corning). Measurements were made at room temperature (~19–23°C).

pH from 8.0 to 7.0 (Fig. 3e). The same proportion of sensors had an internal resistance, determined by the voltage divider technique, in the 2–12 MΩ range (Fig. 3f). There was, however, no correlation ($r < 0.1$) between electrode response time and internal resistance. Additional work is now underway to further evaluate the impedance characteristics of these membranes using a gain and phase analyzer (Solartron Instruments, 1260A) with a frequency range between 10 μHz and 32 MHz.

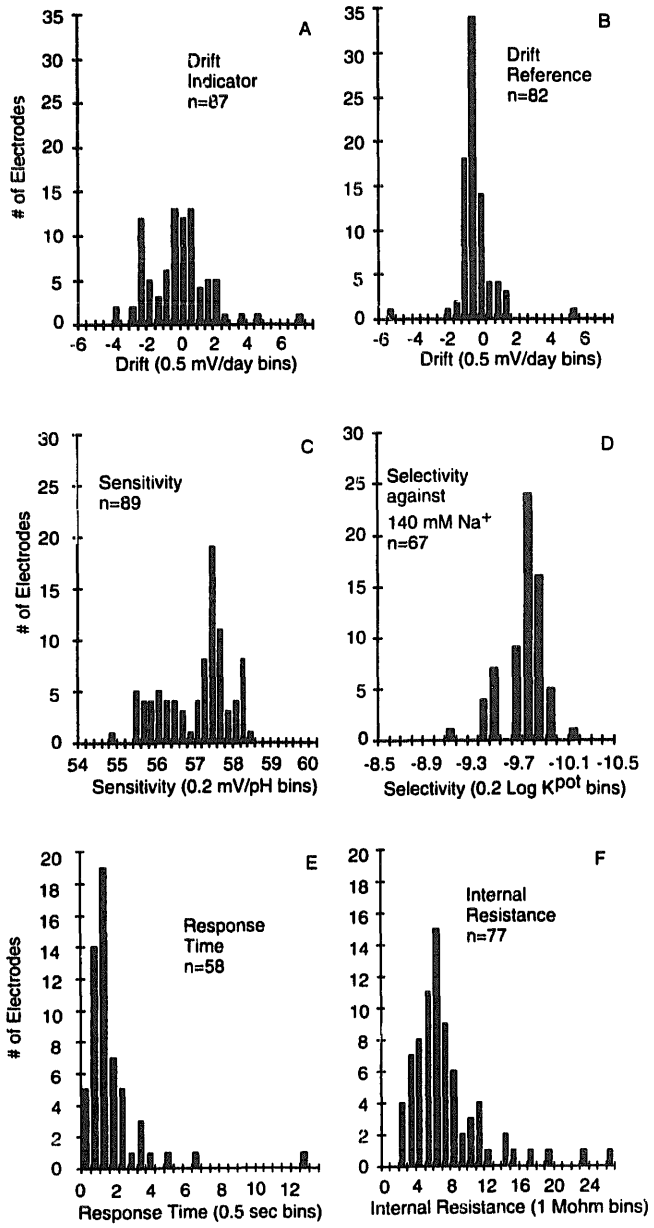

Fig. 3. Histograms for (a) indicator drift, (b) reference drift, and (c) sensitivity, (d) selectivity, (e) response time, and (f) internal resistance of the indicator electrode. Measurements were made prior to implantation in animals. Drift and sensitivity were measured in conjunction with a commercial single-junction Ag/AgCl reference. Response times and selectivities were measured using our own reference electrodes. Internal resistance was determined in conjunction with a Ag/AgCl wire.

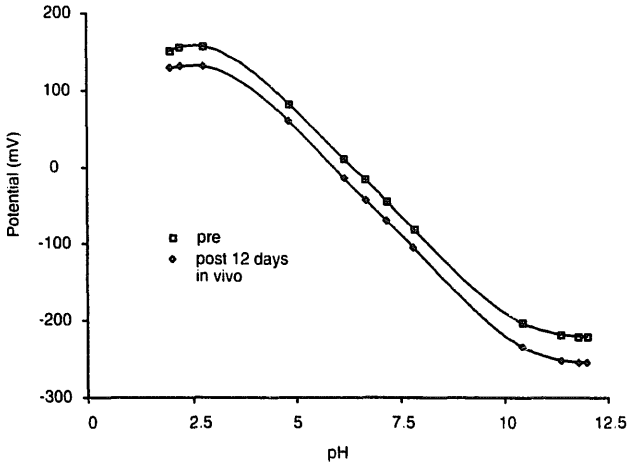

Fig. 4. Titrations between pH 2 and 12 before (pre) and after 12 days subcutaneous implantation. The electrochemical cell was completed with one of our small p-HEMA frit reference electrodes.

3.2. Postimplant

On the whole, the electrochemical measurements described above were changed only slightly following up to 21 days of subcutaneous implantation in rats. Of the 42 indicator and 42 reference electrodes implanted, only 3 (1 indicator and 2 reference) failed completely, although additional electrodes were actively destroyed (e.g. chewed) by the animals during the implant period. Because the PVC membranes were cast on PVC tubing, ion selective electrodes were physically very robust and, unlike previous studies [3], membrane adherence to the electrode body was not a problem.

Electrode sensitivity and selectivity changed very little following implantation, and the changes that were observed were also found in control electrodes. Calibration curves were virtually indistinguishable from those generated prior to implantation, except for a slight baseline shift (e.g. Fig 4). Mean sensitivity measures at 4 and 8 days were not different from pre-implantation values (Fig. 5a), and by 21 days, were only reduced 6–7% (significant at $P < 0.001$). Similar losses in sensitivity were found in control electrodes stored in aqueous buffer solutions at 35°C. Mean selectivity remained, for the most part, unchanged throughout the implantation period, although there may be a very slight reduction (2–3%) by 21 days (Fig. 5b).

The most obvious change observed following implantation was a downward shift or drift in the response baseline (e.g. Fig 4). This drift was due almost entirely to drift of the indicator electrode; our reference electrode showed little change in potential following implantation (Fig. 5c). Moreover, most of the drift in the indicator electrode occurred during the first 4 days of implantation (Fig. 5d), and was temporally correlated with changes in the internal resistance, and to some extent response time, of the

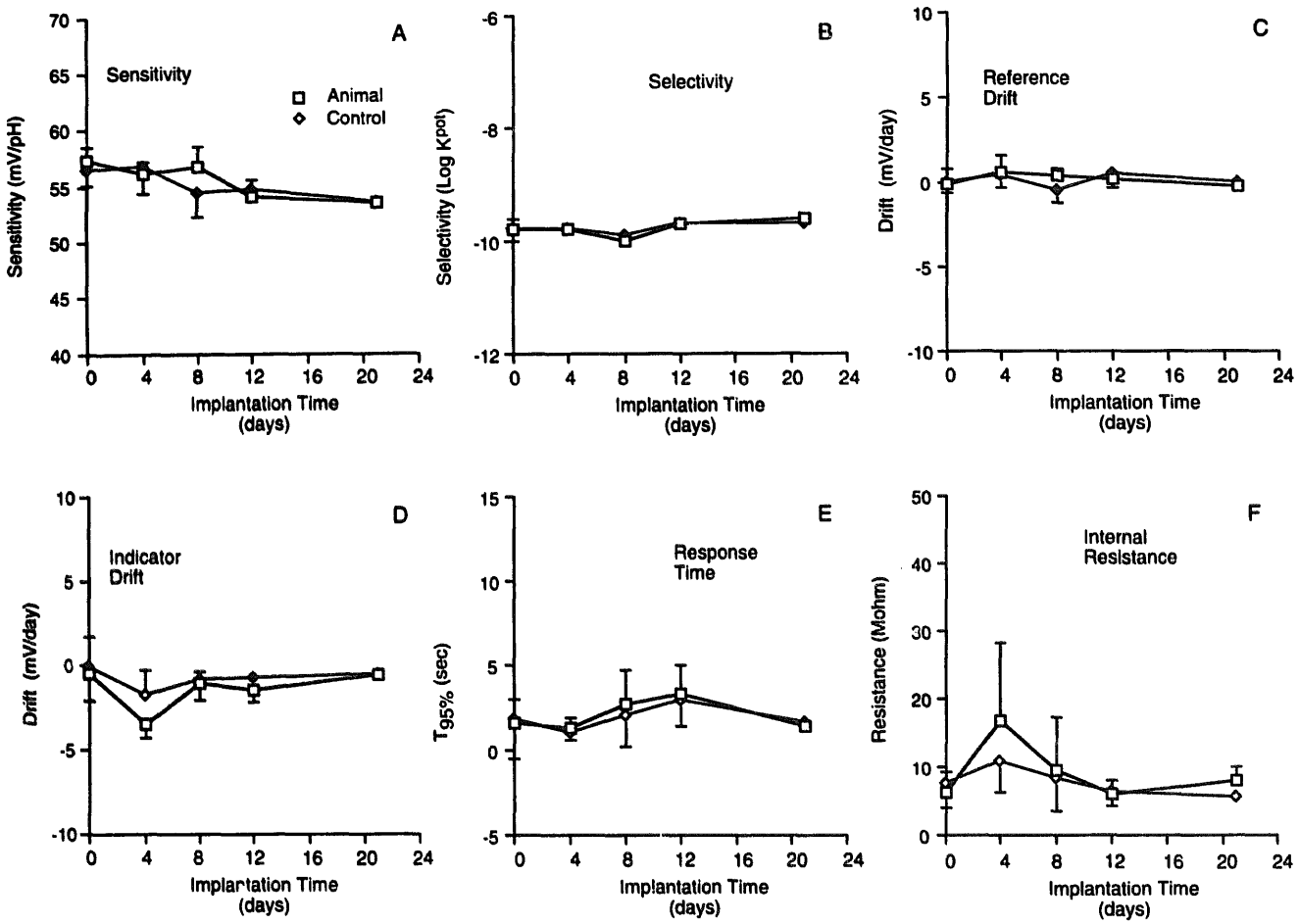

Fig. 5. Post-implant (squares) and control (diamonds) time histories of mean indicator (a) sensitivity and (b) selectivity, (c) reference drift and (d) indicator drift, and indicator (e) response time and (f) internal resistance.

indicator electrode (Fig. 5e,f). Again, similar changes were observed in control electrodes stored at 35°C. Notably, all three measures, drift, response time, and internal resistance return to approximately pre-implant levels by 21 days.

4. Discussion

Based on the results above, we conclude that, with the exception of drift, ion selective, PVC membranes retain much of their capacity for accurate electrochemical measures following as much as 21 days of direct and continuous exposure to rat subcutaneous tissue. The changes that do occur are relatively small and are observed in control (35°C) as well as implanted electrodes.

The reason for the 6–7% loss in sensitivity following 21 days of implantation is unclear. However, since similar losses were found in control electrodes, the decrease is probably related to temperature and electrode age rather than direct tissue effects on the membrane. One possibility is that our membranes are losing plasticizer over time. Decreases in sensitivity are known to occur with reduced plasticizer content [15], and it may be that in our electrodes, especially at elevated temperatures, plasticizer is being lost, either into the sample (or tissue), or into the PVC phase of the electrode body. However, the high lipophilicity of the membrane components should limit the loss of plasticizer into the sample providing lifetimes much longer than 21 days [11]. Likewise, the plasticizer in the electrode body will limit any net loss of plasticizer from the membrane into the electrode body. Thus, loss of plasticizer is probably not the source of the observed sensitivity decrease.

A more likely possibility is that the ionophore concentration in the membrane is decreasing. Again, because of the highly lipophilic nature of the membrane, it is unlikely that significant ionophore is lost to the sample or tissue during a 21-day period. However, ionophore may migrate into the electrode body and decrease the ionophore concentration in the membrane. Reducing ionophore concentration below a critical level is known to decrease both sensitivity and selectivity, while not affecting membrane resistance [11]. This is similar to what we observe at 12–21 days of implantation, suggesting our sensitivity decrease may be due to a diffusion of ionophore into the electrode body. Changes in electrode construction which limit the potential for diffusion of the ionophore out of the membrane should improve the long-

term stability of sensitivity and selectivity with implantation.

The basis of the changes in drift, response time, and internal resistance with implantation are also unknown. In absolute terms, these changes are all relatively small, and with the exclusion of drift, will not significantly impact the general performance of the electrode. They are not associated with direct tissue effects, since similar changes are observed in control electrodes. They do appear related to transient changes in the membrane and not other parts of the electrode, since reference electrodes, which are fabricated almost identically, do not show similar drift. Notably, all of these measures return close to pre-implant values by 21 days, suggesting that long preconditioning times at elevated temperatures might be used to eliminate transient electrode potential drift and changes in response time.

5. Conclusions

In summary we have shown that ion selective, solvent PVC membranes retain most of their capacity for electrochemical analysis following chronic exposure to a physiologic environment. The small changes that do occur are not due to direct tissue effects, but rather appear related to transient membrane changes superimposed on ionophore loss into the plasticized PVC electrode body. This implies that improved electrode construction coupled with extended preconditioning times at elevated temperatures will allow chronic implantation in physiologic systems with little or no change in electrode properties, at least within the time frames studied here. These studies are part of the development of a telemetric chemical sensor system [14] to support the space life sciences research needs of NASA and the human fetal monitoring needs of UCSF's Fetal Treatment Center.

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