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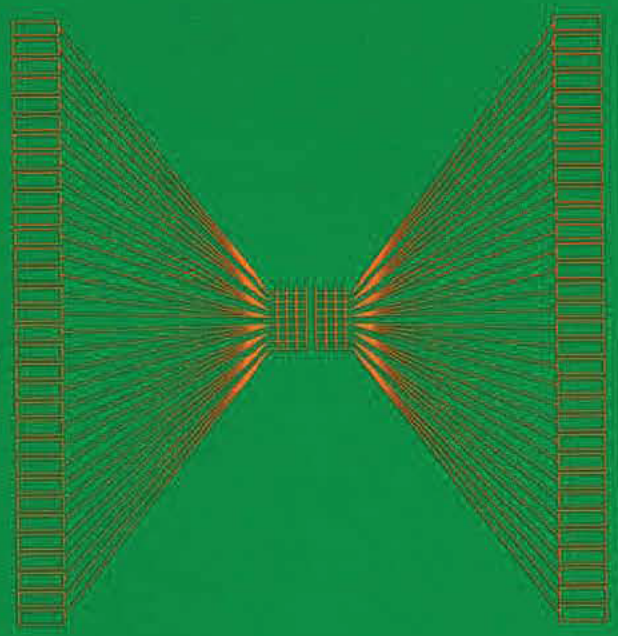
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BIOSENSORS & BIOELECTRONICS

The principal international journal devoted to research, design, development and application of biosensors and bioelectronics

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BIOSENSORS & BIOELECTRONICS

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Rise in background current over time in a subcutaneous glucose sensor in the rabbit: relevance to calibration and accuracy

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Abstract

In order to calibrate a continuous glucose monitor, accurate determination of the background current (I_0) is necessary, in part because I_0 could change over time. We compared two methods of I_0 measurement: (1), extrapolation of sensor output data (as afunction of glucose leve) th the intercept at zero glucose and (2) direct measurement of the output of a blank anode with no enzyme coat. We implanted telemetric sensors subcutaneously in rabbits and measured their outputs during tri-level glucose clamps once per week for 5 weeks. The two methods yielded similar results. I_0 rose substantially over time and this increase reached significance during week 3 by the direct method but not until week 5 by the extrapolation method. Using the direct method, I_0 rose from 3.41 (0.60–8.48 nanoamperes (nA), median and range) during week 1 to 13.42 (9.1–14.3) during week 5. Using the extrapolation method, I_0 rose from 0.57 (0–16.7) during week 1 to 15.3 (12.2–21.6) during week 5. We conclude that I_0 can rise over time. If this rise went undetected and was assumed to be stable, a one-point calibration procedure would overestimate glycemia in the hypoglycemic range, i.e. fail to appreciate the severity of hypoglycemia. It is recommended that during validation of a chronic glucose sensor, I_0 be measured sequentially over time. © 2000 Elsevier Science S.A. All rights reserved.

Keywords: Glucose sensor; Implantable; Electrochemical; Background current

1. Introduction

The Diabetes Control and Complications Trial demonstrated that in persons with Type 1 diabetes, disease of the kidneys, eyes, and nerves can be delayed or reduced in severity by reducing the degree of hyperglycemia (Diabetes Control and Complications Trial Research Group, 1993). Recently, similar findings were reported in a large, well-designed, long-term study in persons with Type 2 diabetes (Prospective Diabetes Study Group, 1998; Turner, 1998). However, achievement of optimal glycemic control remains elusive because intensive therapy carries with it an increased risk of severe hypoglycemia. Furthermore, even with frequent self-monitoring of blood glucose using current (intermittent) technology, glycemic control remains abnormal in most individuals with diabetes (Diabetes Control and Complications Trial Research Group, 1993; Prospective Diabetes Study Group, 1998).

It has been widely acknowledged that continuous monitoring of blood glucose would enhance glycemic control (Gough and Armour, 1995) and would be beneficial by providing early warning of impending hypoglycemia (Aussedat et al., 1997). We recently reported the use of a chronic, telemetric, subcutaneously-implanted sensor in dogs. Sensors typically functioned for approximately 1 month. However, fluid collections developed around many sensors and in many cases prematurely reduced duration of function (Ward and Troupe, 1999).

Accurate prediction of glycemia by an implanted continuous glucose sensor requires accurate calibration of the device. Accuracy is especially important in the hypoglycemic range because failure to detect hypoglycemia can have serious consequences. Calibration schemes generally consist of obtaining the sensitivity and the background current of the sensor at measured blood glucose levels in vivo, then using a regression

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equation to predict future glycemic levels. In a hydrogen peroxide-detecting sensor, background current is thought to result from oxidation of interferants present in interstitial fluid (Zhang et al., 1994). The purpose of this study is to compare the two methods of measuring background current and to assess whether background current can change over time.

In this study, to obtain background current, we utilized the method of extrapolating sensor output (as a function of glucose level) to the y-intercept where glucose is zero. We also examined a different method, the use of a blank indicating electrode which was not coated with glucose oxidase.

In order to accurately measure the background current by extrapolation, we obtained stable sensor output data at each of three glucose levels for each weekly study. Others have demonstrated that there are transient perturbations in the relationship between serum glucose and interstitial glucose during rapid rise and fall of glucose such as that which occurs during oral, parenteral, or intraperitoneal administration of glucose (Thome-Duret et al., 1996). In an attempt to avoid these perturbations, we performed this study at stable glucose levels during the multilevel clamp experiments. We also avoided general anesthesia because of known effects of anesthesia on carbohydrate metabolism (Halter and Pflug, 1980; Diltoer and Camu, 1988).

These methods allowed us to address the question of whether background current changes over time. Our study suggests that, at least under some conditions, background current is not entirely stable and can be found to rise substantially over several weeks.

2. Materials and methods

2.1. Animals and catheter placement

This study was approved, prior to its performance, by the Legacy Health System Institutional Animal Care and Use Committee and by the Legacy veterinarian. Experiments were performed in female New Zealand white rabbits weighing 3.6–4.8 kg (Western Oregon Rabbit Co., Philomath, OR 97370). Rabbits were given laboratory chow and sterilized water ad libitum and were housed in a room with a 12-h light–dark cycle at a temperature of 21.0 ± 1.0°C.

In order to allow multiple blood withdrawals from, and repeated glucose delivery to, conscious animals, chronic indwelling inferior vena cava (IVC) catheters were surgically implanted prior to sensor experiments, as follows: hand-fabricated polyethylene/silicone catheters were inserted into the inferior vena cava during a laparotomy under halothane-induced general anesthesia. Each catheter was then passed posteriorly through the body of the psoas muscle to the dorsal subcutaneous space. The catheter was then tunneled in a superior direction and eventually was exteriorized in the posterior neck. The protruding catheter was protected by a cylindrical pillbox attached to the skin with stainless-steel sutures. The catheter lumen was plugged with stainless-steel stylets and the animals recovered.

2.2. Sensor fabrication

Two widely separated glucose sensors, each of which included an enzyme-coated indicating electrode and an accompanying blank indicating electrode (without an enzyme coat) were placed in each housing disk (see Fig. 1). A single common Ag/AgCl reference electrode (cathode) in each disk served all four of these indicating electrodes (anodes). Each disk housing, made of baked epoxy resin with rounded outside edges, had a diameter of 50.8 mm and a height of 15.2 mm (Resdel Corp., Rio Grande, NJ 08242). The disks were scribed with three concentric rings with diameters of 8, 32, and 36 mm. The two outside rings were machined to a depth of 1 mm and width of 0.3 mm. Material internal to the inner ring was completely removed to create a central cavity for the cathode. Four electrode wiring holes, 0.8 mm in diameter, were drilled into the outside rings. Platinum foil (Sigmund Cohn Corp., Mount Vernon, NY 10553) was cut by UV YAG laser (BioElectric Corp., Portland, OR 97224) to dimensions of $1 \times 25 \times 0.1$ mm and was positioned in the outside rings.

The electrodes were internally soldered to 30 AWG Kynar-insulated silver/copper alloy wires (Alpha Corporation, Elizabeth, NJ 07207). Reference electrodes were machined from silver rods (diameter = 6.35 mm, Alpha Aesar, Ward Hill, MA 01835) to fit the inner cavity (dimensions: 1.3×6.35 mm). Then, 30-gauge Kynar-coated wires were soldered to one side of the silver disks. The platinum strips and the silver disks were permanently bonded into position with a two-component epoxy resin (EP42HT Master Bond Inc, Hackensack, NJ 07601). The dimensions of the exposed platinum electrodes were 0.1×25 mm. The epoxied disks were allowed to cure for 72 h at 40°C. The silver and platinum electrodes were then sanded on a flat surface, under running water, with progressively finer sandpaper up to 2000 grit, and finally polished with a tack cloth. The finished sensors were then checked for electrical continuity through all electrodes. Silver chloride was generated on the central silver disks by a 1.2 mA current through a solution of 1.5 M KCl for 15 min.

After chloridization, the surfaces were flushed several times with a cleaning solution of three parts iso-

propyl alcohol, two parts acetone, and one part deionized water (DW). A glucose oxidase enzyme solution (35 mg glucose oxidase (GOX, Sigma G-6125), 14 mg bovine serum albumin (BSA, Sigma A-4378) dissolved in 230 μl DW, 10 μl 100% isopropyl alcohol, and 100 μl of 25% glutaraldehyde) was applied via a stationary fiber wick while the disk rotated at 100 RPM on an electronic spin-coater (P6204-A, Specialty Coatings Systems, Indianapolis, IN 46241). In this fashion, approximately seven units of GOX was applied to each of the outer two platinum electrodes in each housing thus creating the active glucose sensors. The two inner blank indicating electrodes were left uncoated for the purpose of measuring background current (see Fig. 1 for sensor diagram). The sensors were allowed to dry for 30 min in room air and then immersed in DW for 15 min. Immersion for 15 min was then repeated twice in fresh DW followed by drying with an N_2 stream. The sensors were then heated in an oven at 40°C for 30 min. While maintaining this temperature, the sensors were rotated at 2000 RPM on the spin-coater. Three milliliters of warmed BBF polyurethane (Polymer Technology Group, Berkeley, CA 94610) were then applied directly over the spinning disks. Immediately following application, the rotation was ramped down to 300 RPM for 5 min. A protective membrane consisting of 0.2–3.0 μm expanded PTFE (Millipore Corp., Bedford, MA 01730) was then stretched over the disk surface and secured in place with an O-ring around the side wall.

2.3. In vitro testing and pre-implant preparation

Sensors were soaked overnight in phosphate buffered saline (PBS) with a pH of 7.4. Sensors were tested in a thermostated (37°C) water bath (VWR 13309-385) while data was read from an eight-channel electrometer (Biogen, Portland, OR 97214) connected to a 16-channel data acquisition board (DAP 1200A/4, Microstar Laboratories, Bellevue, WA 98004). Data was recorded in ASCII format on an IBM-clone personal computer. The Biogen electrometer delivered a bias voltage of −0.6 V to the reference electrode. Prior to testing, sensors were immersed in PBS and allowed to equilibrate for 2 h. Data was then recorded in five ascending standards of glucose (G) (D-(+)-Glucose, Sigma G-7528) from G0 to G24 mM. The sensors that qualified for implant were rinsed with DW, dried with N_2, then stored in bell jars until needed. After the transmitter and battery pack were inserted (see below), each disk housing was sealed with a re-enterable sealant (Scotch-Cast 2112, 3M, St. Paul, MN 55104) and the disk capped with a baked epoxy cover. Sensors were sterilized with ethylene oxide and implanted subcutaneously in the left flanks of rabbits.

2.4. Implant specifications

A disk qualified for implant if both of its sensors satisfied three criteria: (1) when placed in G6, no more than 2 min elapsed before they reached 90% of its

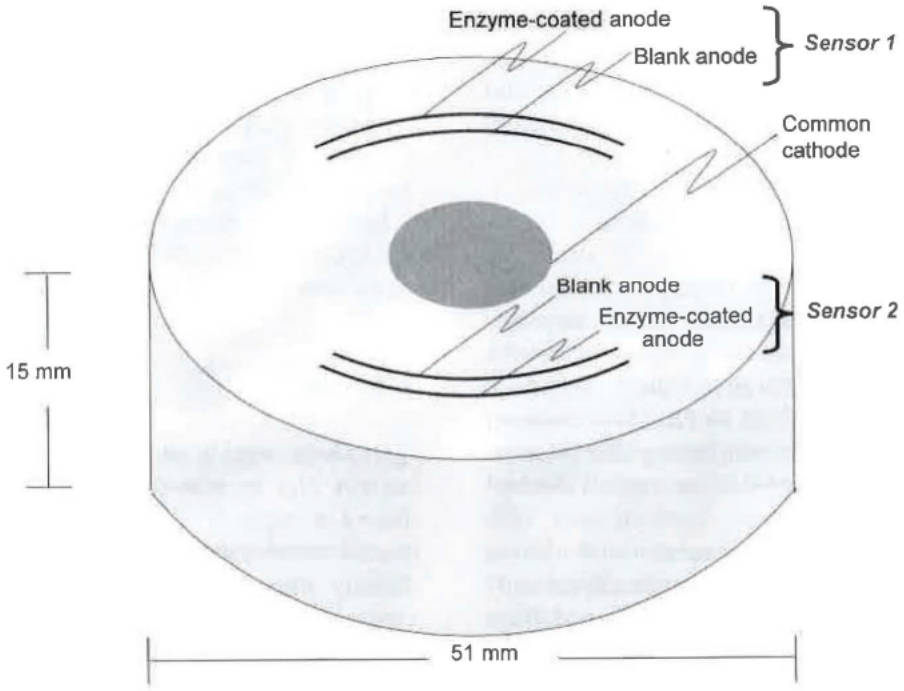


Fig. 1. Disk configuration: two widely separated enzyme-coated sensors, each with a an accompanying blank uncoated anode, are contained in each housing. Each blank anode was separated by 2 min from its accompanying enzyme-coated anode. Contained in the interior of the housing is a transimpedance amplifier, battery pack, and a pulse-period modulated transmitter.

maximum response at that level; (2) they were at least *nearly* linear between GO and G24 as defined by < 33% loss of slope as the sensor is tested in each successively higher standard (this is a more sensitive test of linearity than calculation of the correlation coefficient) and (3) their sensitivity was between 4 and 6 nA/mM between G6 and G24. Since our experience suggests that in vitro data does not accurately predict in vivo performance, no attempt was made to obtain in vivo calibration by using pre-implant in vitro data.

2.5. Data transmission and collection

Each active glucose sensor and each blank electrode had its own specific telemetry transmission channel, i.e. four channels per disk. One disk sensor unit was implanted in each rabbit, thus each rabbit had two active glucose sensors and two blank indicating electrodes. Data was telemetered via trans-impedance amplified transmitters (Minimitter Corp., Sunriver, OR 97707) which had been inserted into the disk housing. Transmission occurred on an FM frequency of 150–152 MHz. The telemetry system functions by varying the transmitted pulse period (from 0.45 to 1.4 s) according to the input originating from the sensor. Before implantation and after explantation, the telemetry circuitry was tested by calibrating it against a series of resistors of increasing value. The resistor values are chosen to correspond to sensor outputs that are generated over the physiologic range of glucose (0–30 mM). The calibration regression is typically an excellent fit with r^2 of > 0.99. During studies in vivo, we calculate sensor current outputs from transmitted periods by applying the regression equation. Data was retrieved by TR-2 telemetry receivers (Telonics, Mesa, AZ 85204) and uncoded/sorted with Datacol 4 Tm data acquisition systems (Minimitter).

2.6. In vivo testing and experimentation

Variable rate glucose infusion (clamp) studies were performed once per week and each lasted approximately 120 min. The first clamp study was performed 1 week after implant. Studies continued until the sensor response was no greater than I nA or electrical circuitry failed. To minimize extraneous electromagnetic interference, the rabbits were studied while in a small double-walled Faraday cage.

Rabbits were conscious and unrestrained during these studies. Baseline sensor data was obtained for 30 min. One-milliliter blood samples were obtained from the venous catheter at $t = 10$, 20, and 30 min and the serum was analyzed in duplicate, after microcentriflagation, using a Beckman glucose analyzer (Beckman Instruments, Brea, CA, 92621). A syringe pump (PHD2000, Harvard Apparatus, Holliston, MA 01746) then delivered a dextrose load and variable rate maintenance infusion of 15% dextrose according to a previously published algorithm Ward et al., 1986 designed to maintain a glucose level of 11–13 mM. Whole blood samples (0.5 ml) were obtained from the venous catheter at $t = 32$, 34, 37, 40, 45 and 50 min in order to adjust the infusion rate. Samples were analyzed at these times using a single drop applied to the reagent strip of a portable hand-held glucose meter (Sure-Step, Lifescan/Johnson and Johnson, Milpitas, CA95035). One-milliliter blood samples were also obtained from the venous catheter at $t = 60$, 70, and 80 min and the serum was analyzed using the Beckman analyzer. At $t = 80$ min, another loading dose and higher maintenance infusion were initiated in order to clamp glucose at 16–18 mM. Samples were taken at $t = 82$, 84, 87, 90, 95, 100 min for portable glucose meter assay and at 110 and 120 min for Beckman glucose assay. The mean of the final three Beckman readings at each of the three glucose levels, and the simultaneous sensor outputs, were used for calibration calculations.

2.7. Data analysis

Data were imported into a statistical and plotting program (SigmaPlot 4.01, SPSS Inc., Chicago, IL 60611) and converted from mV to nanoamperes (nA) by Ohm's law. Background current was calculated in two ways: direct I_0 was taken as the mean of the continuous blank electrode output over the 120-min study. Extrapolated I_0 was calculated by first obtaining the regression line relating the three enzyme-coated sensor outputs plotted against the three stable glucose levels (the mean of the Beckman values for each clamped level), then extrapolating that line back to the y- (sensor output) axis where glucose equals zero. y-Intercepts < 0 were deemed equal to 0. In vivo sensitivity was calculated as the slope of this regression line.

Statistical testing was performed using unpaired Wilcoxon (Mann–Whitney) two-tailed tests. Significance was taken as being less than 0.05.

3. Results

In these weekly studies, we calculated background current (I_0) in non-diabetic rabbits by extrapolation during a triple level glucose clamp using an enzyme-coated anode, and compared these data with I_0 as directly measured by use of an uncoated anode. A representative example depicting the protocol is shown in Fig. 2. It can be appreciated that sensor current rises commensurate with the glucose rise, and that the blank uncoated anode output remains low during the variable rate glucose infusion. Some lag is apparent but since only the final time period at each level is used for

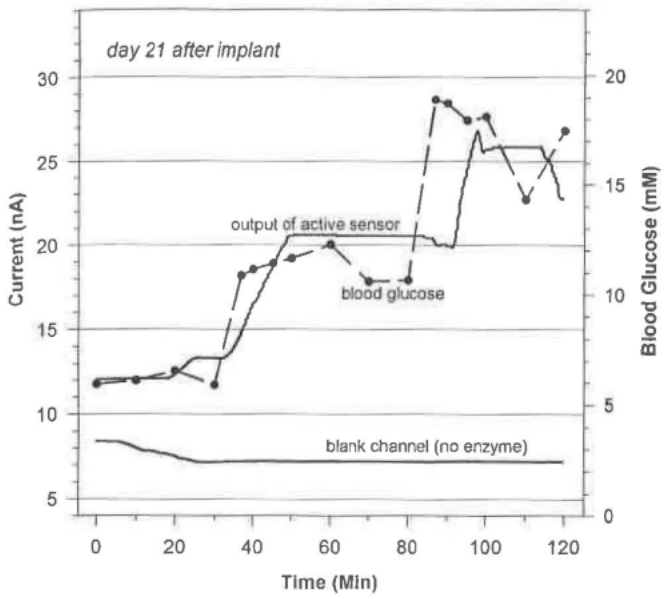


Fig. 2. Depiction of in vivo study protocol showing blood glucose levels and transmitted outputs of an active (enzyme-coated) subcutaneous glucose sensor and its blank counterpart. Data shown was obtained during a weekly hyperglycemic clamp study in a representative rabbit. The blank channel is characteristically unreactive. The three final blood glucose determinations at each level, and the simultaneous sensor outputs, are used for calibration purposes. For this reason, the sensor response lag does not reduce the accuracy of calibration.

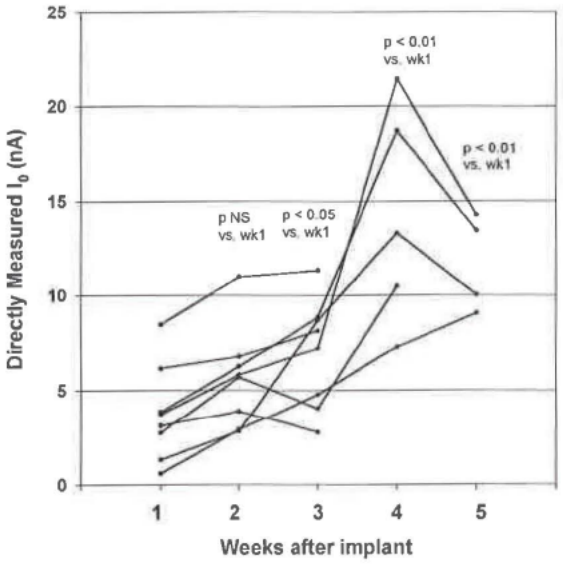


Fig. 3. Directly measured background current (I_0) using a Pt electrode without glucose oxidase: individual data.

calculations, the lag does not hinder accuracy of calibration.

3.1. No discernible 'crosstalk' in the blank electrode

A properly functioning blank electrode must not respond to glucose. Even if otherwise well-designed, a 'blank' electrode could respond to glucose if it were located too close to the enzyme electrode. Such a location could cause it to respond to diffused, unoxidized hydrogen peroxide originating from the active anode, a phenomenon which has been termed chemical crosstalk. To rule out this possibility, we analyzed the blank current during the hyperglycemic clamp experiments and found that this current at the highest glucose level never rose by more than 2.5% as compared with the baseline period. This finding indicates that there no discernible crosstalk in this sensor configuration in which the blank anode was located 2 mm away from the enzyme-coated anode.

3.2. Directly measured I_0 rises over time

Over the course of the study, direct I_0 and extrapolated I_0 increased over time (see individual values for direct measurements in Fig. 3 and mean/SEM values for direct and extrapolated measurements in Fig. 4). The rise in directly measured I_0 reached significance at the 0.05 level during week 3 and 4 and at the 0.01 level during week 5. The rise in the extrapolated measurement did not reach significance (0.05 level) until week 5.

Several points can be appreciated from Fig. 4. The direct and extrapolated methods are similar in that both reveal a rise in I_0 over time. However, they are different in that the central tendency is greater and the error bars narrower for the direct measurements. The greater consistency of the direct method likely accounts in part for its ability to detect the rise in I_0 sooner than the extrapolation method.

There was some evidence for skewness of the extrapolated I_0 data. For this reason, median and range are presented as follows for direct and extrapolated data: week 1: 3.41 (0.60–8.48 nA) and 0.57 (0–16.7, $n=8$); week 2: 5.76 (2.81–10.98) and 0.38 (0–15.8, $n=8$); week 3: 7.68 (2.73–11.3, $n=8$) and 6.74 (0–16.6); week 4: 13.31 (7.3–21.4) and 12.8 (0–31.3, $n=5$); week 5: 13.42 (9.1–14.3) and 15.3 (12.2–21.6, $n=4$).

Sensor sensitivity to glucose, calculated as the slope of the regression line relating sensor output to glucose level, averaged 2.01 ± 0.34 nA/mM for week 1 ($n=8$ for weeks 1–3); 2.45 ± 0.74 for week 2; 1.38 ± 0.64 for week 3; 1.77 ± 0.68 for week 4 ($n=5$); and 1.86 ± 0.43 for week 5 ($n=4$). Sensor sensitivity tends to vary over time in these long-term studies, no attempt was made to use prior sensitivity readings to predict glucose on the basis of sensor output.

After explantation, transmitters were calibrated against a series of resistors. These calibration curves were essentially identical to pre-implant curves. These data demonstrate that it is unlikely that transmitter problems could have accounted for unit failure or for the change in background current.

4. Discussion

The background current (I_0) of an electrochemical sensor is the low level output which is a response to background interferants as opposed to the analyte of interest (in this case, glucose). Many biological compounds in addition to hydrogen peroxide, including ascorbic acid, acetaminophen, uric acid, and probably many others, can be oxidized at a platinum anode polarized at 0.6 V.

In this 5-week study of a chronically-implanted telemetric glucose sensor in rabbits, we simultaneously compared two methods of obtaining the I_0. One method was the oftperformed method of extrapolating the regression line for sensor output measurements to the y-axis where glucose is zero (extrapolated I_0). The other was a direct measurement of the output of a separate anode which was not covered with a glucose oxidase layer (direct I_0). This blank anode was located in the same housing and was the same width and length as the enzyme-containing anode.

We observed that these two methods of measuring I_0 generally give similar results but that it is probably more reliable to measure I_0 directly. For example, over the 5-week study period, we observed a steady rise in I_0. With the use of the direct I_0 measurement, a significant rise was detected in week 3 but the extrapolated I_0 method did not detect the rise until week 5. As can be appreciated by Fig. 4, the variability in the extrapolated I_0 was greater than that of the direct I_0. It should be noted that when one uses several points on a regression line to calculate the y-intercept, error in each of those points will contribute to error of the extrapolated y-intercept. In contrast, measurement of the current from an indicating electrode uncoated with enzyme is inherently a simpler method.

We are not aware of prior reports demonstrating a rise in I_0 over time in electrochemical glucose sensors and its exact cause remains unknown. We believe that a possible source of the rising I_0 may be a slow biodegradation of the outer polyurethane membrane, which may increase permeation of interfering electroactive compounds. Anderson and colleagues have convincingly demonstrated that polyether-based urethanes (such as the one we used in this study) can degrade in vivo over time (Schubert et al., 1995; Stokes et al., 1995). There was also a slight tendency towards non-linearity in vivo over time as can be seen in Fig. 5. Given the fact that the outer membrane serves to retard glucose entry and increase oxygen entry, a degradation of this membrane would be expected to reduce linearity as a result of increasing oxygen deficit. We have previously observed that a reduced thickness of the outer permselective polyurethane membrane leads to non-linearity of the sensor response (Ward et al., 1994).

The rise of I_0 over time has implications pertaining to the methodology of sensor calibration. In situations wherein I_0 is not repeatedly measured over time, a slow rise will not be detected. Of course, not all sensors would be expected to undergo a rise in I_0; I_0 would be expected to be lower and perhaps more stable in sensors with special membranes designed to exclude interferants (Csoregi et al., 1994; Moussy et al., 1994a). In such sensors, direct measurement of I_0 may be helpful

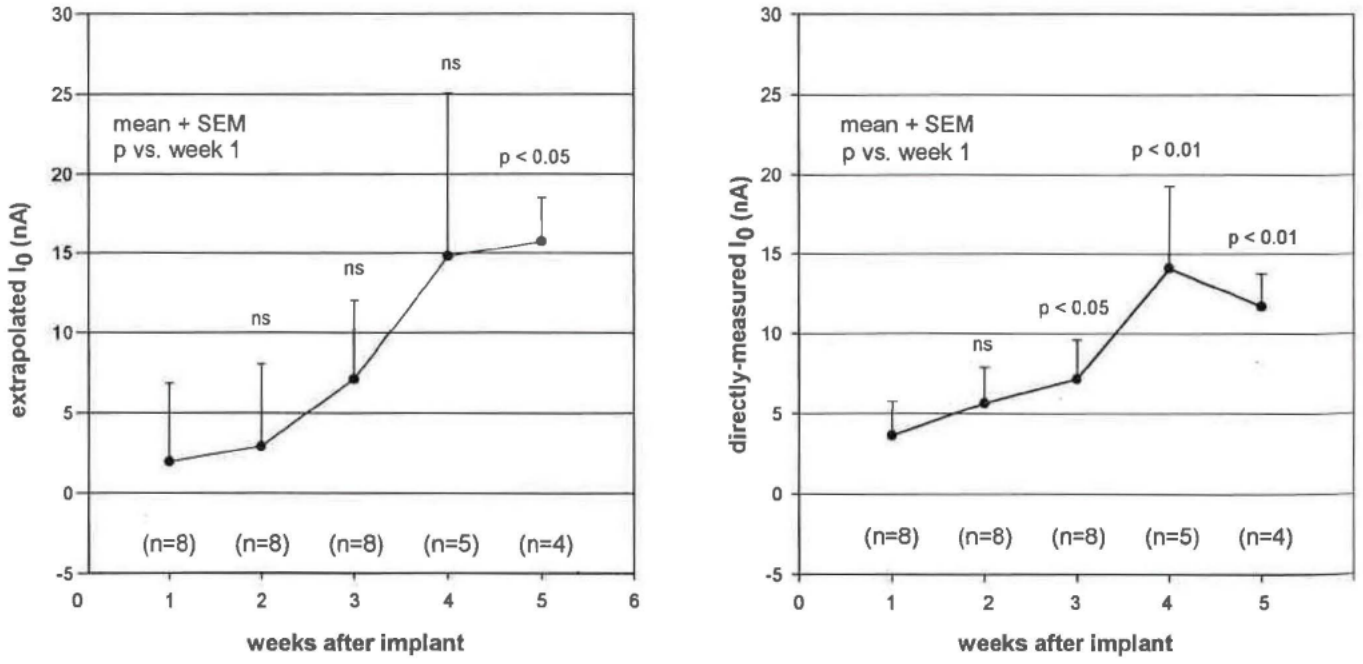


Fig. 4. Extrapolated I_0 (left panel) versus directly measured I_0 (right panel) in rabbits. Extrapolated I_0 data does not yield a significant difference from week 1 to week 5. In contrast, direct I_0 obtained in all weeks from weeks 3 and 5 is significantly higher than in week 1.

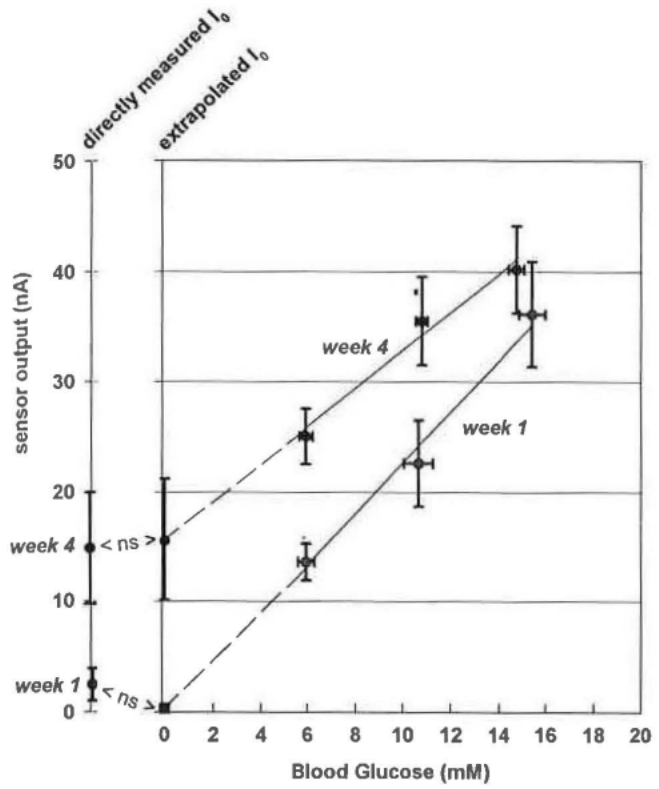


Fig. 5. Depiction of the three clamped glucose levels and the extrapolation process for weeks 1 and 4. Data (mean ± SEM) are shown for the five sensors which functioned for at least 4 weeks. On the vertical axes, measured I_0 is compared with extrapolated I_0.

to rule out degradation of the anti-interference membrane over time. In glucose sensor systems where I_0 comprises a substantial portion of the sensor output current (Pickup et al., 1993), accurate measurement of I_0 assumes an even greater importance.

We used a sequential three point variable rate glucose infusion method in order to accurately predict the in vivo dose–effect curve. This clamp method is quite cumbersome, time-consuming, and requires intravenous access (obtained in our study by the use of chronic inferior vena cava catheterization). This multiple clamp method is certainly not feasible for routine clinical utility. Nonetheless, holding glucose steady at basal and two elevated levels provides near steady-state conditions which minimizes glucose estimation error which could otherwise result from rapid transients and sensor measurement lags. A multi-level clamp also allows for accurate in vivo estimation of linearity which is not possible with a one-point or two-point calibration. Thus, when developing an electrochemical glucose sensor, it might be reasonable to use a steady-state multiple glucose clamp method for initial definition of dose–effect assessment and validation of linearity in a small number of subjects before allowing routine use of less cumbersome calibration methods. It is essential that strict linearity be obtained when one-point or two-point calibration schemes are used because non-linearity will create error in both.

Other investigators have also examined the issue of background current and its measurement. Like us, Harrison et al. have fabricated a glucose sensor with a non-enzyme-coated anode which is used in a pairwise fashion with an enzyme-coated anode for in vitro testing (Harrison et al., 1988). This pair-matched system was not used in the group's in vivo studies, however (Moussy et al., 1994b).

Many groups have estimated I_0 by extrapolation in vivo without use of a blank anode. For example, the Paris/Kansas group, who like us use a peroxide-detection system, report extrapolated I_0 obtained from a two-point calibration (Aussedat et al., 1997). This methodology is similar to the method used by Fischer et al. in dogs (Fischer et al., 1994). Shichiri et al. did not report specific I_0 values during in vivo studies and did not incorporate a blank anode in the design of their electrochemical sensor. But they may not have needed an independent measure of I_0 given the fact that they did not need an in vivo calibration for the first 7 days then were able to use a one-point calibration for the ensuing 14 days (Shichiri et al., 1998). For their in vivo calibration scheme, Gilligan et al. did not use an I_0 measured in vivo and instead used the I_0 which had been measured during in vitro testing. Their design does not include a separate blank indicating electrode (Gilligan et al., 1994). This group from Wisconsin has data suggesting that, after an initial unstable period, sensor function ultimately becomes more stable. Although our results suggest that background current rises over time, it may not be appropriate to extrapolate our results directly to glucose-monitoring systems of others. Differences in device materials, methodology and animal species may also explain some of the differences in results.

Schmidke and Heller report a very low I_0 which allows them to perform a one-point calibration. Their low I_0 is presumably related to their use of a separate membrane which oxidizes interfering substances which otherwise would contribute to the background current (Schmidtke and Heller, 1998). Drs Reach and Wilson and co-workers also use a special inner membrane composed of multiple layers of cellulose acetate and Naflon in order to exclude interfering substances (Moatti-Sirat et al., 1994; Zhang et al., 1994). The Edmonton group has also reported use of special membranes in order to exclude interfering substances. These membranes have been composed of Nafion (Moussy et al., 1994b) or Poly(phenylenediamine) (Moussy et al., 1994a).

Armour and colleagues reported the use of a non-enzyme-containing electrode to measure background current. Although similar to ours in concept, their methodology is not directly comparable with ours in that they measure glucose via an enzyme-mediated de-

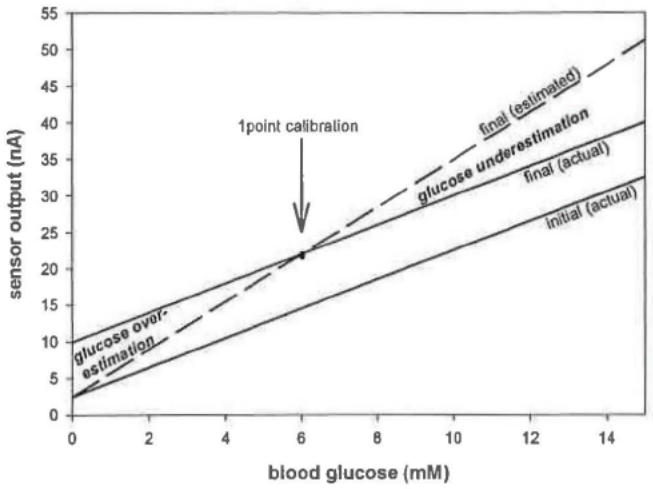


Fig. 6. Expected results of calibrations if there is a failure to detect a rising I_0 over time. In our study, over 4–5 weeks, I_0 rose by 4–5-fold, as illustrated in 'initial (actual)' and 'final (actual)'. If a one-point calibration is utilized but I_0 is assumed to be unchanging over time, as illustrated in 'final (estimated)', one would overestimate glucose for sensor outputs below the calbration level and underestimate glucose for higher outputs. Such a situation could (dangerously) fail to accurately assess the degree of hypoglycemia.

cline in oxygen tension as glucose rises. Their background assessment technique measures oxygen separate from glucose. In their study of prolonged intravenous sensor recording in dogs, they do not recalibrate in vivo but are able to use the original in vitro calibration (Armour et al., 1990).

With regard to methods of sensor calibration, our studies may be relevant to accurate detection of hypoglycemia. Our observed rise in I_0 over time suggests that, in the setting of a chronic implanted glucose sensor, an initial calibration may not be sufficient for the duration of the implant. Fig. 6 shows the effect of failing to detect a rise in I_0 (equal to what we observed) over time in the context of a one-point calibration method. This example shows that the assumption of a constant I_0 will lead to the false assumption that sensitivity has risen and will overestimate glucose in the region where sensor output is lower than the calibration point. The further sensor current falls below the calibration point, the greater the error. Given its dangerous consequences, hypoglycemia must be accurately detected. For this reason, it is important to strictly verify the behavior of calibration variables such as I_0 and sensitivity over time.

In summary, measurement of background current (I_0) using a direct method yielded results generally similar to a calculated, extrapolated I_0. The direct method was superior in that it was able to detect the time-related rise in I_0 earlier in the study. Measurement of I_0 directly using a blank anode may improve calibration accuracy of implantable electrochemical glucose sensors.

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