Influence of inflammatory cells and serum on the performance of implantable glucose sensors

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Received 6 March 2000; revised 23 May 2000; accepted 11 July 2000

Abstract: The objective of this investigation was to evaluate the influence of polymorphonuclear granulocytes on the performance of uncoated and cellulose acetate/Nafion coated amperometric glucose sensors in vitro. The response of these sensors was also investigated in serum. Uncoated and coated sensors showed lower sensitivities to glucose, with a significant drift in sensor output upon exposure to serum or leukocytes. Although the use of a coating resulted in higher sensitivity, the progressive loss of output was not completely prevented. Stimulated granulocytes were shown to excrete components, probably catalase and myeloperoxidase, which consumed the hydrogen peroxide formed by the oxidation of glucose. In addition, adsorbed serum proteins formed a diffusional barrier for glucose. Furthermore, serum was found to contain low-molecular weight components that alone inhibited glucose oxidase activity. Based on preliminary electrochemical results, we postulate that rabbit serum contains oxidizing substrates that compete with molecular oxygen for the acceptance of electrons from the oxidized enzyme. Consequently, future efforts should be aimed at elucidating the mechanisms involved in the interference of unknown serum components with electron transfer. In addition, further investigations have to be performed to develop an outer membrane that minimizes protein adsorption as well as the actions of inflammatory cells. © 2000 John Wiley & Sons, Inc.

Key words: glucose sensor; implantable; inflammatory cells; peroxidases; glucose oxidase; serum; oxidizing substrates

INTRODUCTION

One of the major obstacles in the development of a reliable amperometric enzyme electrode based on glucose oxidase has been the drift in sensor signal after implantation.1–3 In addition to sensors based on hydrogen peroxide detection, mediator-based electrodes also display impaired responses after implantation. Despite satisfactory in vitro performance for prolonged periods of time, it is still not possible to maintain the functionality of an implanted sensor in an in vivo environment. The inflammatory phase of the tissue response to the implanted sensor has been suggested as an important reason for the observed signal drift after implantation.4 In this context, it has been supposed that:

1. Inflammatory cells affect sensor output by local consumption of glucose around the implanted sensor,5,6
2. Cellular attachment to the sensor surface influences glucose diffusion,7,8 or
3. Damage of sensor components occurs by excreted proteolytic enzymes and free radicals.1,8

Nevertheless, no structural research has been performed on the influence of inflammatory cells on this progressive loss of sensor sensitivity.

Recently, Reichert and Sharkawy9 have reported that a caged implant system can provide an excellent
means of assessing the inflammatory response to a functioning sensor in vivo. In addition to examining the effects of protein adsorption and cellular reactions on sensor performance, such a device also allows information to be gathered on the actual diffusion of glucose to the sensor through the fibrous tissue capsule that usually surrounds a sensor. In a previous in vivo experiment, we already showed that a subcutaneously implanted tissue chamber indeed makes it possible to follow glucose kinetics in retrieved tissue fluid. On the other hand, first generation glucose sensors placed in the chamber could not reliably monitor these changes. We know that adsorption of proteins from the tissue fluid to the sensor surface negatively influences glucose diffusion, but we do not know to what extent this was the case in our caged implant system. Furthermore, in this particular experiment, histologic evaluation revealed the presence of high numbers of leukocytes in the subcutaneous chamber. On the other hand, we also observed sensor current stabilization in tissue fluid that was first collected from the subcutaneous chamber. Consequently, we hypothesize that, in addition to protein adsorption, an ongoing cellular reaction to the implanted sensor plays an important role in the process of sensor inactivation.

Therefore, the objective of the current investigation was to study the influence of the cellular reaction on sensor performance in vitro. Uncoated first generation amperometric glucose sensors, based on the detection of hydrogen peroxide, as well as sensors provided with an outer cellulose acetate/Nafion® coating were tested. We already used this type of coating in a previous experiment investigating the response of amperometric glucose sensors in a subcutaneous tissue chamber. Since the polymorphonuclear granulocyte (PMN) is the predominant cell type in the first stages of the inflammatory phase of the tissue response, these cells were used in this study. To further elucidate the mechanisms involved in sensor inactivation, we evaluated sensor response in serum and assessed the influence of leukocytes and serum on the activity of glucose oxidase (GOx).

**MATERIALS AND METHODS**

**Glucose sensor preparation**

The sensor consisted of a platinum wire (diameter 1 mm) and a silver wire (diameter 0.5 mm), placed in a stainless steel tube (o.d. 3.175 mm) and sealed with epoxy resin (Araldite). The probe was polished with lapping film to 0.3 μm and sonicated in water for 1 min. After rinsing with aceton/ethanol, silver/silverchloride paint (Acheson Colloiden B.V., Scheemda, The Netherlands) was applied to the silver wire and allowed to dry at 4°C for at least 1 h. The probe was poised at 1.1 V versus Ag/AgCl for 15 min in 0.1 M HCl and subsequently placed in 0.1 M phosphate-buffered saline solution (PBS, pH 7.4, room temperature), containing 15 mM 1,3-diamino benzene, 15 mM resorcinol, and 4 mg/mL glucose oxidase [E.C. 1.1.3.4] type II (265.8 IU/mg) from Aspergillus niger, Genzyme, Cambridge, USA) for 15 min. Ten scans from 0 to 0.8 V versus Ag/AgCl at a rate of 2mV/s were applied to immobilize the enzyme using an autolab PGSTAT-10 (Eco Chemie, Utrecht, The Netherlands). For obtaining a coated sensor, the probe was additionally dip-coated at 4°C in a 2.5 wt % cellulose acetate solution in aceton/Nafion® (1:1) and stored for 10 min. After drying in 5% Nafion®, the sensor was allowed to dry for 10 min. After fabrication, the sensors were allowed to stabilize for 24 h in PBS. During the measurements, a potential of 700 mV versus Ag/AgCl was applied using an Antec EC Controller or a home built potentiostat. Figure 1 shows a schematic illustration of the coated sensor as used in the experiments.

**Solutions**

Percoll, with a density of 1.076, was prepared by adding 41.8 mL of PBS 10x (90 g/L NaCl, 13.9 g/L NaH2PO4 in aquadest), 220 mL of PBS 1x, and 97.5 mL of pasteurized plasma solution with 0.38% tri-sodium citrate to 500 mL of percoll with a density of 1.130. Hank’s buffered salt solution (HBSS) was prepared by dissolving 0.185 g/L CaCl2·2H2O, 0.40 g/L KCl, 0.06 g/L KH2PO4, 0.10 g/L MgCl2·6H2O, 0.10 g/L MgSO4·7H2O, 8.00 g/L NaCl, 0.35 g/L NaHCO3, and 0.06 g/L NaHPO4·2H2O in Milli-Q with a pH of 7.4. Lysation buffer was made by dissolving 8.30 g/L NH4Cl and 1.0 g/L KHCO3 in Milli-Q with a pH of 7.4. Glucose stock solution was prepared at a concentration of 1 M glucose in Milli-Q. Serum was obtained from healthy New Zealand white rabbits; whole blood was collected by venipuncture from an aural vein in a glass tube. After 30 min of coagulation, followed by centrifugation at 3,500 rpm for 10 min, serum was collected and stored at -20°C. Glucose

**Figure 1.** A schematic illustration of the coated sensor used in the experiments: A is araldite resin, B is reference electrode (Ag/AgCl), C is working electrode (Pt), D is stainless steel, E is glucose oxidase/DAB-resorcinol, F is cellulose acetate/Nafion® coating. The overall diameter of the sensor is 3.175 mm; the diameter of the working electrode is 1 mm and the reference electrode is 0.5 mm.
analysis in serum was performed by an APEC glucose analyzer (Stam Instruments B.V., Tiel, The Netherlands). The reaction mixture for the glucose oxidase activity assay was prepared by dissolving 78 mg 2,2-azino-bis-(3-ethylbenzenothiazolin-6-sulfonzuur)-di ammonium salt (ABTS) and 3 mg horseradish peroxidase (HRP) in 100 mL of HBSS.

Isolation of polymorphonuclear granulocytes

Heparinized whole blood (20 mL) was collected from healthy New Zealand white rabbits and mixed with 10 mL of HBSS, pH 7.4. Subsequently, the mixture was carefully pipetted onto 12.5 mL of percoll (ρ = 1.076) in a polypropylene tube and centrifuged at 2,000 rpm for 20 min at room temperature (Minifuge GL, type 4400, Heraeus-Christ, Ostenrode, Germany). Plasma and the layer containing mostly lymphocytes, monocytes, and platelets were aspirated to within 1.0 cm of the top of the red blood cell pellet and discarded. Contaminated red blood cells were lysed by resuspending the pellet in 50 mL lysis buffer on ice. After centrifugation at 1,500 rpm for 5 min, remaining red blood cells were lysed in 50 mL lysis buffer at 4°C. The final pellet was washed and resuspended in 10 mL of HBSS. Cell viability was determined by trypan blue dye exclusion. With flow cytometry, leukocytes were counted and differentiated. Finally, the concentrated cell solution was diluted with HBSS or rabbit serum to give a final white blood cell concentration of 1 × 10⁶ cells/mL. Cell viability was always greater than 99%, and in all cases the PMN fraction contributed to over 90% of the total number of leukocytes with an average of 95 ± 1%.

Preparation of serum-opsonized zymosan and stimulation of PMNs

Zymosan, 250 mg (Sigma, St. Louis, USA) was suspended in 30 mL of PBS and sonicated at 48 kHz for 1 h. After centrifugation (for 5 min, 400 × g), the pellet was resuspended in 6 mL of rabbit serum and incubated for 45 min at 37°C with continuous shaking. The serum-opsonized zymosan was then washed three times and resuspended in PBS at 10 mg/mL and stored at −70°C. Polymorphonuclear granulocytes were stimulated in solution with serum-opsonized zymosan at a final concentration of 1 mg/mL. The glucose concentration of the zymosan suspension was adjusted by addition of the appropriate amount of 1 M glucose stock solution.

Sterilization

HBSS and the glucose stock solution were sterilized using a filter with a pore size of 0.2 μm (Millipore, Etten-Leur, The Netherlands). Glass tubes were sterilized by autoclave. Uncoated sensors were sterilized in 70% ethanol for 60 min. Cellulose acetate/Nafion® coated sensors were placed in sterile 0.9% saline for 60 min before usage.

Sensor performance

The sensitivity to glucose of both uncoated and coated sensors was determined in HBSS, HBSS containing PMNs, rabbit serum, and rabbit serum containing PMNs. Experiments were performed five times each at room temperature. A potential of 700 mV versus Ag/AgCl was applied. Before each experiment, sensitivity to glucose was determined in HBSS. After stabilization of the background current, glucose was added stepwise to a concentration of 10 mM and the sensitivity to glucose was determined. Subsequently, the sensor was placed in HBSS to allow the current to return to basal values. The sensor was then placed in HBSS with PMNs (10 mM glucose), rabbit serum, or rabbit serum with PMNs. Granulocytes were activated with opsonized zymosan just before placement of the sensor in the test solution. The sensor current was monitored for 3 h. The sensitivity to glucose in the test solution was determined at maximum sensor current and compared with the sensitivity in HBSS. Sensor drift was calculated as percentage of current decrease in the first h of exposure to the PMNs and/or serum. After the experiment, the sensor was placed in HBSS with 10 mM glucose. After 1 h, the sensitivity to glucose was determined and compared to the sensitivity in HBSS before exposure to PMNs and/or serum.

Glucose oxidase activity

The influence of PMN excretion products and rabbit serum on GOx activity was determined spectrophotometrically by following the oxidation of ABTS. Release of excretion products was initiated by the addition of serum-opsonized zymosan (1 mg/mL) to the PMN suspension in glass tubes (1 × 10⁶ cells/mL in HBSS with 5 mM glucose). After incubation at 37°C for 60 min under continuous shaking, the reaction was stopped by placing the samples on ice. Subsequently, the suspension was centrifuged for 5 min at 400 × g, followed by collection of the supernatant containing the excretion products.

The glucose concentration of the test solutions was adjusted to 50 mM to exclude substrate limitation. In addition, air was bubbled through the solutions to prevent oxygen limitation of the enzyme reaction. The reaction was initiated by the addition of 5 μL GOx (0.5 mg/mL) to 1 mL of the test solution. At 0, 5, 10, 15, 20, and 30 min after initiation, 5 μL of these mixtures was added to 1 mL of the reaction mixture containing ABTS and HRP. After 30 min incubation in the dark, the absorbency was determined at a wavelength of 420 nm using a UV-spectrophotometer. The absorbency was plotted against time and a trend line was determined as a measure of ABTS oxidation. Glucose oxidase activity was calculated in international units (I.U.) from the slope of the trend line. The experiment was performed in duplicate. To investigate hydrogen peroxide consumption by granulocyte excretion products, we followed absorbency at a constant hydrogen peroxide concentration of 1 μM in the absence of GOx. Sodium azide (1 mM) was added to investigate the
role of heme-containing peroxidases like myeloperoxidase and catalase. The same procedure as described above was followed.

RESULTS

Sensor performance

Table I shows the properties of the uncoated and coated sensors in HBSS. Sensor current was shown to be stable with an average sensitivity of 17.6 ± 4.1 nA/mM for the uncoated probes. The sensitivity of the coated probes was significantly lower, averaging 1.1 ± 0.5 nA/mM. Again, these sensors showed no drift in output in HBSS.

Table II shows the characteristics of the uncoated and coated probes in HBSS with PMNs, rabbit serum, and rabbit serum with PMNs, i.e., their sensitivity during and after exposure as a percentage of the sensitivity in HBSS, and the drift in sensitivity as percentage decrease of initial sensitivity in the first h of exposure. The sensitivity of the uncoated sensors in the presence of PMNs was lower than in HBSS alone, 71.0 ± 7.1%. In serum this was even lower, 45.7 ± 7.9%, while in serum with PMNs the sensitivity to glucose again was lower compared to serum alone, 32.2 ± 8.2%. Statistical analysis, using a one-way analysis of variance (ANOVA) with multiple comparison (Student’s-Newman-Keuls), showed that all these differences were significant (p < 0.001). For the coated sensors, comparatively higher sensitivities were observed. In the presence of PMNs, sensitivity to glucose was 83.9 ± 11.8%, in serum 70.9 ± 13.8%, and in serum with PMNs 67.4 ± 16.4%. This time, these differences were not statistically significant (p > 0.05). The differences in sensitivity between uncoated and coated sensors for all test solutions were statistically significant (Student’s t test, p < 0.05).

The current of uncoated sensors did not stabilize in the presence of PMNs, serum, or serum with PMNs during the 3 h of exposure. A drift in output of 23.1 ± 10.1% was observed in the presence of granulocytes. In serum the drift was 36.9 ± 2.7%, while in serum containing PMNs a drift of 47.6 ± 7.2% was noticed. Statistical analysis revealed that there was a significant difference in current drift in the presence of both serum and granulocytes compared to serum or PMNs alone (one-way ANOVA with Student’s-Newman-Keuls, p < 0.05). The output of coated probes almost completely stabilized in the presence of leukocytes or serum after 3 h. However, when both serum and leukocytes were present this phenomenon did not occur. A drift of 7.4 ± 1.7% was observed in salt solution containing PMNs. In serum, the drift output was 12.9 ± 5.4%, while in the presence of both leukocytes and serum it was 24.0 ± 3.5%. Again, the differences in current drift between the test solutions were statistically significant for the coated sensors (one-way ANOVA with Student’s-Newman-Keuls, p < 0.01). Figure 2 shows representative examples of the drift of an uncoated and coated sensor observed in serum with leukocytes.

In all experiments, for uncoated as well as coated sensors, a return in sensitivity was observed in HBSS after exposure. However, sensitivity was not completely restored. Remaining sensitivity of our uncoated probes was 84.3 ± 2.4% in the PMN solution, 83.8 ± 15.6% in serum, and 79.2 ± 15.1% in serum containing PMNs.

### Table I

<table>
<thead>
<tr>
<th>Characteristics of Uncoated and Cellulose Acetate/Nafion® Coated Sensors</th>
<th>Uncoated Sensors (n = 15)</th>
<th>Coated Sensors (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity to glucose (nA/mM)</td>
<td>17.6 ± 4.1</td>
<td>1.1 ± 0.5</td>
</tr>
<tr>
<td>Baseline current (nA)</td>
<td>5.3 ± 2.7</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>Linearity</td>
<td>&gt;15 mM</td>
<td>&gt;15 mM</td>
</tr>
<tr>
<td>Response time</td>
<td>Range &lt; 1 min</td>
<td>Range 2–5 min</td>
</tr>
</tbody>
</table>

### Table II

<table>
<thead>
<tr>
<th>Characteristics of Uncoated Sensors in Buffered Salt Solution (HBSS) with PMNs, Rabbit Serum, and Rabbit Serum with PMNs</th>
<th>Uncoated Sensors</th>
<th>Cellulose Acetate/Nafion® Coated Sensors</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBSS/PMN</td>
<td>Serum</td>
<td>Serum/PMN</td>
</tr>
<tr>
<td>Sensitivity in test medium as percentage of sensitivity in HBSS</td>
<td>71.0 ± 7.1</td>
<td>45.7 ± 7.9</td>
</tr>
<tr>
<td>Drift in sensitivity in the first h of exposure to test medium as percentage of initial sensitivity</td>
<td>23.1 ± 10.1</td>
<td>36.9 ± 2.7</td>
</tr>
<tr>
<td>Remaining sensitivity in HBSS 1 h after exposure to the test medium as percentage of initial sensitivity in HBSS</td>
<td>84.3 ± 2.4</td>
<td>83.8 ± 15.6</td>
</tr>
</tbody>
</table>

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containing leukocytes. For the coated probes, remaining sensitivities were 82.3 ± 4.1% in the PMN solution, 69.8 ± 8.7% in serum, and 66.2 ± 15.6% in serum containing leukocytes. No significant differences in remaining sensitivity were observed after exposure to leukocytes, serum, or serum with leukocytes for both uncoated and coated sensors (one-way ANOVA, \( p > 0.05 \)). In addition, no differences were demonstrated between both probe types for any of the test solutions (Student’s \( t \) test, \( p > 0.05 \)).

Glucose oxidase activity

Table III shows glucose oxidase activity in HBSS, in the presence of PMN, and in rabbit serum. Enzyme activity was significantly lower in the presence of granulocyte excretion products. Ultrafiltration (molecular weight < 1 kDa) completely prevented this decrease in reaction rate. In rabbit serum, a significant decrease in ABTS oxidation was observed. This time, ultrafiltration (molecular weight < 1 kDa) was not shown to have a positive effect. Figure 3 shows the absorbency at a constant concentration of hydrogen peroxide in buffered salt solution. PMN excretion products were shown to convert hydrogen peroxide, as is illustrated by the sharp decline in absorbency. Again, ultrafiltration (molecular weight < 1 kDa) completely reversed this phenomenon. Also, the addition of sodium azide (final concentration 1 mM) prevented the occurrence of hydrogen peroxide consumption.

DISCUSSION

Some critical remarks have to be made about the design of our study. We determined the drift in sensor output after 1 h of exposure to granulocytes and serum. This could be determined because we knew that in vitro the generation of reactive metabolites is self-limiting and nearly complete within 1 h after the addition of an activating stimulus.\(^ {14} \) On the other hand, after subcutaneous implantation, sensors are subject to a sustained attack of leukocytes and thus continuously exposed to their secretory products. The same is probably true for the process of biofouling, i.e., the adsorption of serum proteins. In vitro, serum causes an initial rapid drop in sensor activity that tapers off and approaches steady state after several h.\(^ {9} \) Nevertheless, in the much more complex in vivo environment, the kinetics and severity of this process are likely to be different. Despite these structural differences in reaction

![Figure 2](image-url) Relative decrease of sensor output (I/I\(_0\)) of an uncoated (lower line) and a coated sensor (upper line) in the presence of serum and granulocytes. A drift of approximately 40% was observed for the uncoated sensor, while the coated probe showed a drift in output of approximately 20% in the first h of exposure. In both cases, sensor current did not stabilize in the 3 h exposure period.

![Figure 3](image-url) Graph showing the absorbency at a constant hydrogen peroxide concentration of 1 \( \mu \)M in buffered salt solution (HBSS), in buffered salt solution with granulocyte excretion products (PMN), its ultrafiltrate (PMN < 1 kDa), and after addition of sodium azide (PMN/azide). In the presence of granulocyte excretion products, a sharp decrease in hydrogen peroxide concentration was observed. This phenomenon was not present after ultrafiltration or addition of sodium azide.

<table>
<thead>
<tr>
<th>Test Solution</th>
<th>Glucose Oxidase Activity (I. U.)</th>
</tr>
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<tbody>
<tr>
<td>HBSS</td>
<td>0.203</td>
</tr>
<tr>
<td>PMN</td>
<td>0.056</td>
</tr>
<tr>
<td>PMN &lt; 1 kDa</td>
<td>0.231</td>
</tr>
<tr>
<td>Rabbit serum</td>
<td>0.056</td>
</tr>
<tr>
<td>Rabbit serum &lt; 1 kDa</td>
<td>0.055</td>
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</table>

The depicted value is the average of two separate measurements.
mechanisms, we think that the current study design can still provide information on failure mechanisms of implantable sensors in a biological system.

It has been recognized that the in vitro behavior of a sensor after exposure to a biological environment can often reveal the source of bioinstability. The fast return of sensitivity of our sensors after exposure to leukocytes or serum is regarded as indicative of a lack of oxygen preventing glucose oxidation, electrochemical interference, or a reversible inhibition of peroxide oxidation at the sensor electrode. Oxygen limitation of the enzyme reaction can be excluded in our study, since an experimental set-up with test tubes warrants diffusion of adequate quantities of oxygen. Nonspecific interference from electroactive species can also be excluded because this is prevented by the use of di-amino benzene resorcinol.

Our results clearly demonstrate the negative influence of granulocytes on sensor performance. In the glucose oxidase activity assay we showed that granulocytes excrete substances that cause a significant decrease in reaction rate, predominantly through hydrogen peroxide consumption. Ultrafiltration revealed that these substances have a molecular weight over 1 kDa. Furthermore, the addition of sodium azide almost completely prevented this inhibitory effect. Since we know that azide inhibits the activity of heme-containing enzymes, we think that this phenomenon is mainly caused by catalase or myeloperoxidase excreted by granulocytes. In additional experiments using SDS PAGE, distinct bands of approximately 49,000 and 62,000 daltons were detected in the excretion products of stimulated granulocytes. These are probably indicative of the presence of myeloperoxidase and catalase, respectively. These findings support the previously mentioned suggestion of a reversible inhibition of peroxide oxidation as a source of sensor instability. Our observation that a cellulose acetate/Nafion® coating prevented this negative effect for the greater part, illustrates that an optimized outer membrane can minimize the negative influence of leukocytes on sensor performance. Consequently, based on the current information, the following conclusion appears to be justified: The partial loss of sensor sensitivity after exposure to granulocytes is probably caused by the adsorption of proteolytic enzymes and other macromolecules excreted by these cells to the sensor surface. In view of this, we have to emphasize that damage to glucose oxidase itself by proteolytic enzymes or free radicals seems unlikely as an explanation for the loss of sensitivity. This is especially true because we know that this enzyme is very resistant to proteolysis.

Evidently, protein adsorption played an important role in the observed decrease in sensitivity of our sensors in serum. The application of a cellulose acetate/Nafion® coating resulted in significantly less decrease in sensitivity to glucose and drift in output. These observations can be explained by the physico-chemical nature of the process of protein adsorption. Immediately upon contact with serum, the surface of the sensor becomes coated with a layer of proteins, followed by a competitive exchange of different proteins. This is known as the Vroman effect. The formation of such a protein layer is known to inhibit glucose diffusion to the sensor. The final thickness and composition of this layer determine the extent to which diffusion is inhibited. Because this is dependent on the characteristics of the sensor surface, it will be clear that the improvement in response of coated sensors can be attributed to the use of the coating. Furthermore, partial desorption of proteins from the sensor surface in buffered salt solutions explains the incomplete return of sensitivity of our sensors after exposure to serum. On the other hand, the decrease in reaction rate in the glucose oxidase activity assay was shown to be completely caused by low-molecular weight species. This indicates that protein adsorption was not the only mechanism that caused the failure of our sensors in serum. Glucose oxidase activity measurements in serum revealed inhibition of the enzyme reaction by low-molecular weight serum components. Sensor inactivation through this phenomenon has also been demonstrated by others. Still, the exact mechanisms have not yet been elucidated. This negative influence has been related to inhibition of enzyme activity or reversible fouling of the electrode. Inhibition of peroxide oxidation through electrode fouling can be excluded in our situation because this process has been shown to be prevented by the application of di-aminobenzene and resorcinol. The possibility remains that inhibition of glucose oxidase activity is caused by unknown serum components. Several inhibitors are known from literature, but to what extent these actually inhibit enzyme activity in rabbit serum is still unknown. Therefore, we would like to suggest another explanation for the decrease in sensitivity and drift of our amperometric sensors in serum. It has been reported that, in addition to molecular oxygen, glucose oxidase can be oxidized by a variety of other electron acceptors. This concept has been employed in second-generation amperometric glucose sensors for the transfer of electrons from enzyme to electrode. If serum contains unknown oxidizing components that compete with molecular oxygen for the acceptance of electrons from the oxidized enzyme, a reduction in sensor current will occur through decreased formation of hydrogen peroxide. We already know that serum contains large quantities of powerful oxidants such as quinones. In other organisms possessing glucose oxidase, these quinones are the natural substrate for the enzyme. Quinones also have the same pH optimum as oxygen. In view of this, it appears that other electron acceptors present in rabbit
serum are also able to oxidize glucose oxidase. In fact, cyclic voltammetry in serum ultrafiltrate (molecular weight < 1 kDa) confirmed the presence of unknown oxidants with reduction potentials in the range of ~300 to ~400 mV versus SCE. Currently, research efforts are directed towards the structural characterization of these oxidizing substrates in serum.

In summary, we conclude that both serum and polymorphonuclear granulocytes have a negative effect on the performance of amperometric glucose sensors. Stimulated granulocytes were shown to excrete components, probably catalase and myeloperoxidase, which consumed the hydrogen peroxide formed by the oxidation of glucose. In addition, adsorbed serum proteins formed a diffusional barrier for glucose. Furthermore, serum was found to contain low-molecular weight components that by themselves inhibited glucose oxidase activity. Based on preliminary electrochemical results, we would like to postulate that rabbit serum contains oxidizing substrates that compete with molecular oxygen for the acceptance of electrons from the oxidized enzyme. Consequently, future efforts should be aimed at elucidating the mechanisms involved in the interference of unknown serum components with electron transfer. In addition, further investigations have to be performed to develop an outer membrane that minimizes protein adsorption as well as the actions of inflammatory cells.

References