

# A Subcutaneous Glucose Sensor With Improved Longevity, Dynamic Range, and Stability of Calibration

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**OBJECTIVE** — To evaluate the lifetime, response time, linearity, glucose range, and calibration stability of two different types of continuous glucose sensor implants in a dog model.

**RESEARCH DESIGN AND METHODS** — Glucose sensors based on the enzyme electrode principle that are coupled to a radio transmitter were evaluated on the bench top, sterilized, and then implanted subcutaneously in nondiabetic mongrel dogs. A multichannel radio receiver and PC data processor were used to record the sensor glucose data. Initial early reliable sensor responsivity was recognized by a vigorous hyperglycemic excursion after an intramuscular injection of glucagon. Periodically, the dogs were made temporarily diabetic by blocking pancreatic insulin secretion by subcutaneous injection of a synthetic somatostatin (octreotide). By using exogenous insulin injection followed by intravenous glucose infusion, glucose levels were manipulated through the entire clinical range of interest: 2.2–38.9 mmol/l (40–700 mg/dl). Every 5–10 min, reference blood glucose samples were obtained and run in our hospital clinical laboratory. The glucose sensor data was evaluated by linear least squares optimization and by the error grid method.

**RESULTS** — Beginning as early as postimplant day 7, the *in vivo* performances of sensors were evaluated by using glucose infusion studies performed every 1–4 weeks. Bench-top and *in vivo* 90% response-time sensors were in the range of 4–7 min during sensor lifetime. Best-performing sensors from both types are summarized as follows. The earlier-stage technology was less linear with a dynamic range of no more than 22 mmol/l glucose, had a best-case recalibration interval of 18 days, and had a maximum lifetime of 94 days. The improved later-stage technology sensors, which were constructed with the addition of bioprotective and angiogenic membranes, were linear over the full extended range of clinical interest (2.2–38.9 mmol/l [40–700 mg/dl glucose]), had a best-case recalibration interval of 20 days, and had a maximum lifetime of >160 days.

**CONCLUSIONS** — Stable clinically useful sensor performance was demonstrated as early as 7 days after implantation and for a sensor lifetime of 3–5 months. This type of subcutaneous glucose sensor appears to be promising as a continuous and painless long-term method for monitoring blood glucose. Specifically, sensors with top-layer materials that stimulate angiogenesis at the sensor/tissue interface may have better dynamic measurement range, longer lifetimes, and better calibration stability than our previously reported sensors.

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Abbreviations: FBC, foreign body capsule; PC, IBM-compatible computer.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

The Diabetes Control and Complications Trial (1) and the U.K. Prospective Diabetes Study (2) demonstrated that tight control of blood glucose reduced the risk of the complications of diabetes. To achieve this tight control safely, a painless and continuous method of monitoring glucose over the long term is needed. Our approach to developing this technology is to implant a sensor subcutaneously based on the enzyme electrode principle (3–5). A comprehensive review of this work as well as other subcutaneously implanted glucose sensors was recently published (6). To be clinically useful and practical, the sensor must be easy and safe to implant, provide accurate long-term real-time blood glucose estimates, and provide previously unavailable trend information. The sensor must be coupled to a readout device that includes high and low alarm functions. Furthermore, the sensor implant must be manufacturable and easy to sterilize. In this report, we demonstrate such a device in a dog model.

## RESEARCH DESIGN AND METHODS

Two types of sensor implants were obtained from Markwell Medical (Racine, WI). The first type involved early device development and has been previously described (4,5,7). In this design, a polyurethane membrane with entrapped glucose oxidase is applied with an O-ring over a polarographic H<sub>2</sub>O<sub>2</sub> sensor. The sensor responds linearly to glucose concentrations from 2.2 to 22.2 mmol/l (from 40 to 400 mg/dl) even when oxygen is at a low physiological tension of only 40 mmHg (7). In addition, as shown in Fig. 1, a bioprotective membrane covers the sensing membrane layers. However, the outermost angiogenic membrane is not present.

The bioprotective layer functions to prevent macrophages from gaining close proximity to the enzyme active membrane (5,8). Macrophages orchestrate the formation of the foreign body capsule (FBC) and are known to undergo degranulation and release of potent (but short-lived) oxidative species from the enzyme myeloperoxidase (5,8,9). For example, this enzyme produces hypochlorite (bleach) that can destroy the

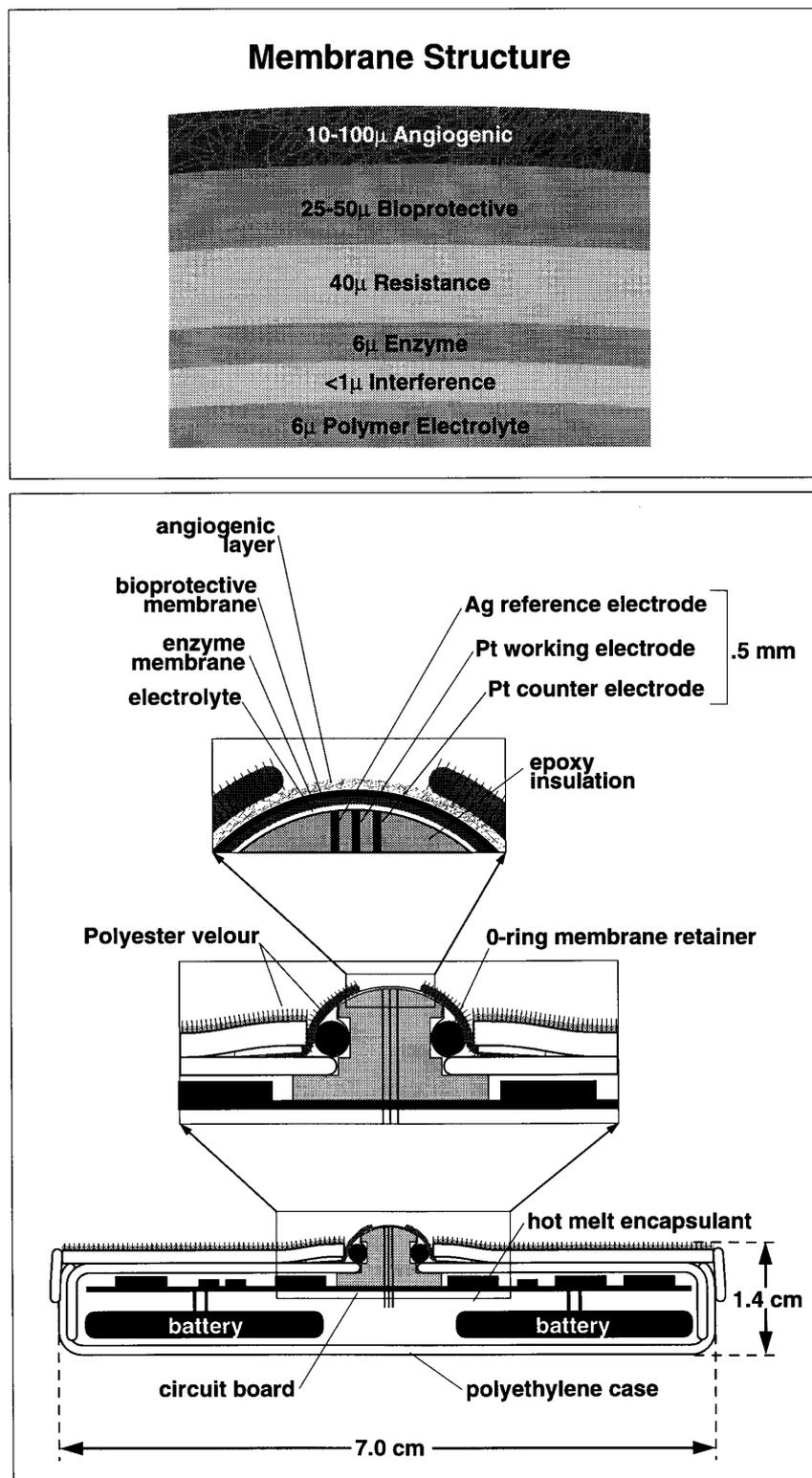


Figure 1—Schematic presentation of sensor membrane layers, electrodes, and supporting electronics.

enzyme active polyurethane membrane and can cause rapid sensor failure unless a bioprotective layer is placed over the enzyme active membrane.

The second type of sensor is identical to the first except for the addition of an angiogenesis layer based on expanded polytetrafluorethylene. This layer was added

over the bioprotective layer to encourage the formation of capillaries adjacent to the sensor. The performance differences with and without this angiogenesis membrane are discussed in RESULTS.

#### Preimplantation bench-top testing and calibration

Before implantation, the sensor is calibrated, and the 90% response time is determined with 5.55-mmol/l glucose concentration steps from 0 to 22.2 mmol/l. An average error of <5% is required to continue the sensor qualification process. Lowering pO<sub>2</sub> from 150 to 30 mmHg shows no more than a 10% drop below perfect linearity in sensor output at 22.2 mmol/l glucose (400 mg/dl), or the sensor is rejected. Stability of calibration must be maintained to within ±15% for a week after the addition of bioprotective and angiogenic membranes to proceed to sensor implantation.

#### Sensor sterilization

Wet sterilization was performed with either 0.05% thimerosal or 2.4% glutaraldehyde for 16 h at room temperature. The sterilant residue was removed by three consecutive 2-min rinses with copious sterile normal saline. This sterilization protocol was performed just before surgical implantation. Sensors were implanted under general anesthesia (sodium thiopental [Pentothal; Baxter Healthcare, Glendale, CA] induction to effect and 1% halothane) into the paravertebral thoracic subcutaneous tissue. The guidelines for the use and care of laboratory animals at the University of Wisconsin were followed.

#### Postimplant sensor evaluation

The enzyme electrode sends glucose data via radiotelemetry every 32 s (for glucose infusion studies) or every 256 s (for long-term drift studies) to a radio receiver multichannel scanner (Pro 2006; Tandy, Fort Worth, TX) coupled to a computer data acquisition interface card. Sensor data is recorded continuously from shortly after implantation to shortly before explantation. The telemetry system and electrode/transmitter packaging have been previously described (10).

#### Sensor readiness

After implantation, FBC formation is needed to establish adequate delivery of glucose and oxygen to the sensor. Glucagon (0.4 mg; Lilly, Indianapolis, IN)

was injected intramuscularly during the first week after implantation to assess whether the sensor was responsive to glucose. If little or no response was evident, then the glucagon challenge was repeated every 2 or 3 days until the sensor responded with the characteristic substantial glucose excursion. Only then did we proceed to full in vivo testing.

#### In vivo testing and calibration

To evaluate the accuracy of the sensor implant through the full clinical range of interest, we injected a subcutaneously synthetic somatostatin, octreotide (Sandostatin [44  $\mu\text{g}/\text{kg}$ ]; Novartis, East Hanover, NJ), that inhibits secretion of insulin, thus making the dog temporarily diabetic (4). Reference blood glucose samples were drawn from a cannulated vein every 5–10 min throughout the 4- to 6-h experiment. Each sample was anticoagulated in lithium heparin test tubes and immediately centrifuged. The plasma fraction was stored on ice until analyzed within 18 h in our hospital clinical laboratory (hexokinase method, Model RxL Dimension; Dade Behring, Newark, DE). After baseline reference samples were obtained, typically regular insulin (0.1 U/kg) was injected intravenously to produce a hypoglycemic excursion. Once blood glucose reached  $\sim 2.2$  mmol/l (40 mg/dl), 10% glucose was infused intravenously through a contralateral cannulated vein after an infusion algorithm designed to produce a straight glucose ramp (11). When the ramp reached the glucose range of 27.5–44.4 mmol/l, the pump was turned off to allow glucose to return to a normal fasting level.

Data analysis comparing the sensor to reference glucose was made by standard least squares techniques and by using the error grid schema introduced by Clarke et al. (12). Sensor versus reference blood glucose data were analyzed retrospectively to obtain optimized calibration factors by using a standard linear least squares technique. For both types of sensor, the maximal useful recalibration interval was estimated by retrospective analysis of data from glucose infusion studies by using a moving average of previous calibrations. To do this, all infusion study data were analyzed and plotted with the Clarke error grid (12). As calibration factors from further back in time are applied to analyze specific glucose infusion studies, the error in plots of sensor glucose versus reference glucose increases. We defined the maxi-

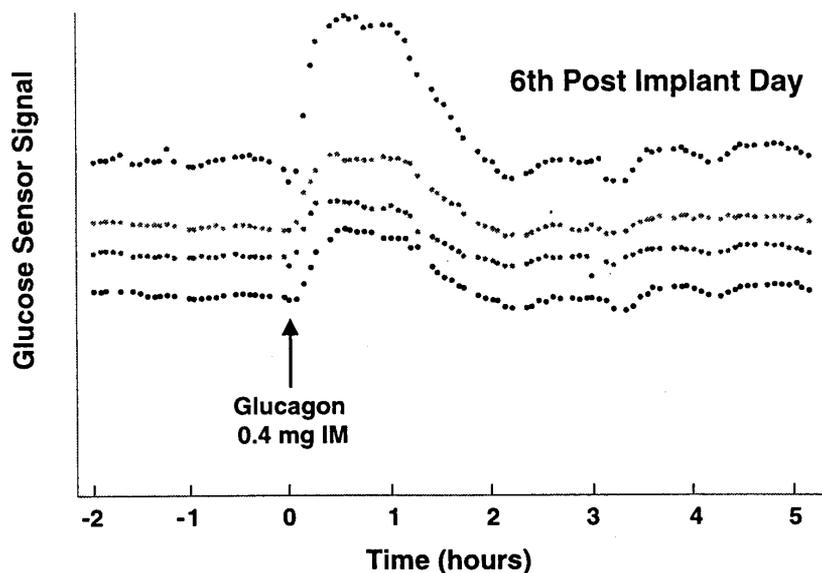


Figure 2—Uncalibrated response to intramuscular injection of 0.4 mg glucagon on day 6 after implantation of four subcutaneous sensors into a single dog.

mum useful recalibration interval as the longest time interval between infusion studies that, when applied across all infusion studies, still permits  $>90\%$  of the calculated versus reference glucose values to remain in the A and B zones of the Clarke grid. This is a useful tool for comparing calibration stability of different sensors.

These definitive in vivo sensor performance evaluations were run every 1–4 weeks until the sensor failed. The sensor was then surgically explanted and retested in vitro, and an attempt was made to reestablish performance in vitro or at least to ascertain the failure mode. The sensor tissue interface was evaluated under the dissecting microscope with hematoxylin and eosin histology.

**RESULTS** — A typical response to glucagon (0.4 mg administered intramuscularly) is shown in Fig. 2 for four simultaneously placed implants on the sixth day after implantation. These implants are all of the second type and use the newer bioprotective and angiogenic membrane materials. Note the highly correlated features of all four sensors, which indicate successful glucose tracking by all four sensors. This type of early behavior, occasionally as early as 1 day after implantation, is typical of most of our recently implanted sensors. The earlier type of sensor without the angiogenic materials that used a slightly recessed sensor geometry often took 10–20 days before this type of glucose response occurred.

Figure 3A shows a typical in vivo glucose infusion curve generated from a recently implanted sensor constructed with both bioprotective and angiogenic membranes. The octreotide was injected to block endogenous secretion of insulin. The intravenous insulin injection rapidly decreased blood glucose into the hypoglycemic range, and then intravenous glucose infusion allowed a slow increase in the blood glucose level to just  $>27.7$  mmol/l (500 mg/dl). The response to glucose was linear, which can be seen from the data replotted as sensor versus reference glucose in Fig. 3B. In this case, a time shift of 5 min was applied to obtain an optimal retrospective calibration and the best correlation with reference plasma and sensor glucose values (correlation coefficient = 0.99, root mean square error = 0.78 mmol/l, average error = 12.9%).

Figures 4A and B present data on the best-performing subcutaneous sensor implant when using the earlier technology, which did not have an angiogenesis membrane (5). Note in Fig. 4A that, when blood glucose increased to  $>16.7$  mmol/l (300 mg/dl), this sensor began to become nonlinear for glucose. This nonlinear behavior is actually due to oxygen limitation in the FBC adjacent to the sensor (4,5). This type of behavior is typical for sensors that do not use the angiogenic outer membrane.

Figure 4A shows a summary plot with a superimposed Clarke error grid for all 10 of the in vivo glucose infusion studies per-

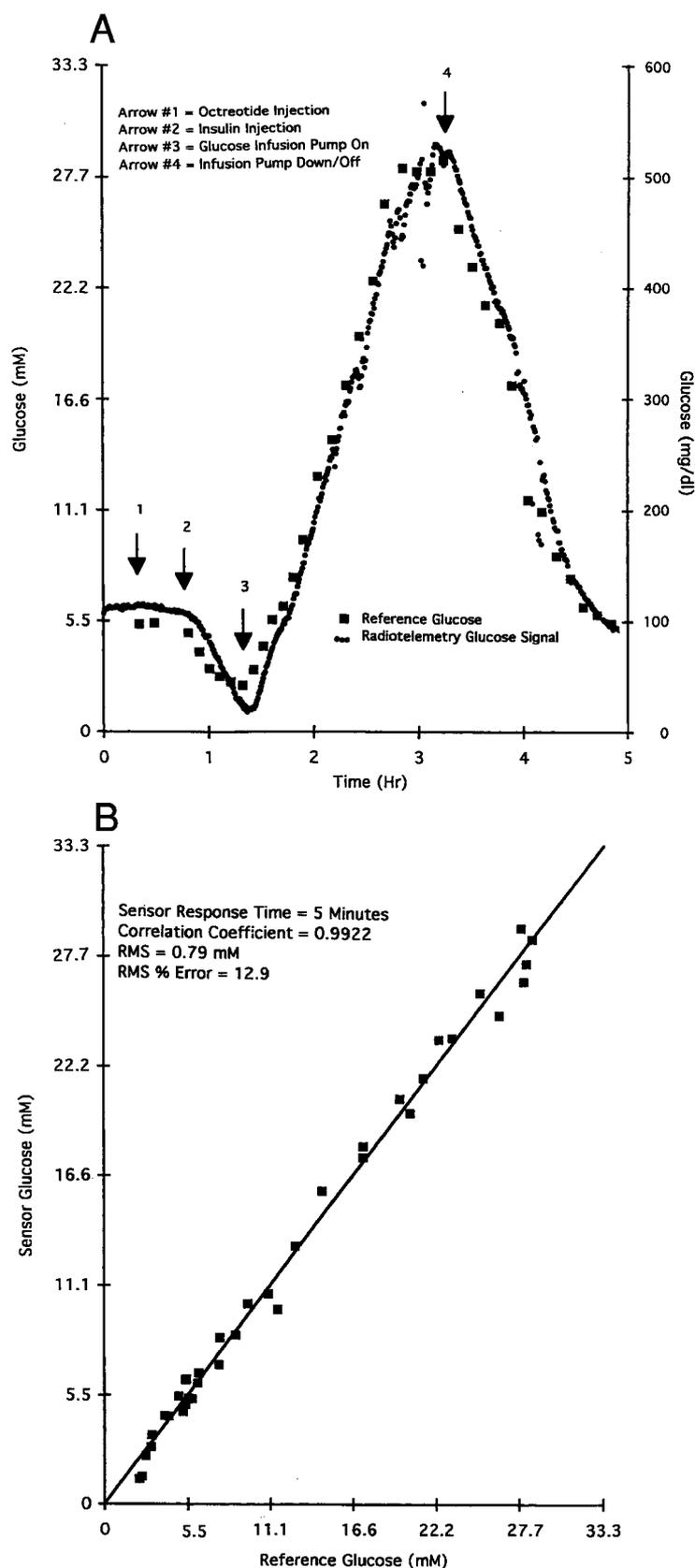


Figure 3—In vivo sensor glucose infusion and calibration study on day 55 after implantation. A: Timing of insulin and octreotide injection and when the glucose pump was turned on and off. B: The linear glucose response through the full range of clinical interest. RMS, root mean square.

formed on this sensor (days 21–94 at 1- to 2-week intervals). A retrospective same-day calibration was used to optimize the sensor to reference glucose values for each day of the study. The Clarke error grid shows 97.9% of the data in the clinically acceptable A and B zones. However, in the clinical setting, comprehensive daily recalibration is impractical. Instead, we envision a sliding or moving average calibration based on previous retrospective calibrations that results in defining more widely spaced recalibration intervals. As an example of this, Fig. 4B shows the same data based on a moving average retrospective recalibration interval of once every 18 days. Note that, although much more scatter to the data is evident in Fig. 4B than in 4A, the Clarke error grid schema still shows 92.1% of the data in the A and B zones. However, if the recalibration interval is further expanded to 26 and then 36 days, then only 87.9 and 77.9% of the data remain in the desired zones, respectively, and this trend in data deterioration increases as the recalibration interval is further widened.

In summary, this particular early type of sensor was vastly superior in performance to others in its class. Typically, one-third of these sensors failed to provide even 2 weeks of glucose tracking. The average sensor lifetime for this class (n = 14) was 39 days, and recalibration intervals of <1 week were typical.

In contrast, Fig. 5A and B show our best sensor implant performance to date for a sensor that includes both bioprotective and angiogenic membrane layers. Note that the linear range of glucose response in Fig. 5A has increased to approximately twice that observed in Fig. 4A. Figure 5A also shows Clarke error grids superimposed on the summary plots of sensor versus reference blood glucose for eight in vivo glucose infusion studies (days 7–162 at 1- to 3-week intervals). When the data are plotted by using the retrospective same-day calibration that optimizes each study, then 95.6% of the data fall into the A and B zones.

To demonstrate the stability of this sensor's calibration, Fig. 5B shows the data for days 21–162 plotted with an average recalibration interval of 20 days. These data points are calculated with calibration factors derived from the previous two infusion studies. For example, the day 21 infusion study used calibration factors derived by averaging the in vitro calibration and the optimized recalibration from the first glucose infusion study on day 7. From then

on, the moving average calibrations were based on the previous two sensor recalibrations obtained from the glucose infusion studies performed 21–54 days previously. This moving average calibration protocol resulted in an average calibration interval of  $20 \pm 6$  days. If the day 7 data are calculated with the in vitro calibration and are included in the data summation, then only 83.1% of the data fall within the A and B zones. This occurs because this day 7 data set with a slightly elevated glucose baseline current (relative to the in vitro baseline) results in all day 7 data being slightly in the C zone. However, if we allow exclusion of this early data and start at day 21 data, then Fig. 5B shows 92.0% of the data in the A and B zones. We consider this to be a reasonable adjustment because some sensors will not have established a stable baseline within the first 10 days after implantation. In addition, calculations for days 21–162 using longer recalibration intervals of 37, 55, and 71 days show only slowly developing spread in the data because 87.1, 84.0, and 82%, respectively, remain in zones A and B. This is much better than that observed for the best of the sensors with the earlier technology.

Thus, the best of the second class of implantable sensors shows significant overall performance improvements relative to the earlier type of sensor without the angiogenesis layer. Additionally, the sensor-to-sensor variability is not as severe as for the earlier class of sensors. Although we are in the fairly early stages of testing this type of sensor ( $n = 6$ ), the average lifetime has increased to  $>105$  days, only one of six sensors has failed to track glucose over multiple weeks, and half of the sensors show recalibration intervals of  $>10$  days. Finally, the histology of these sensors shows consistent thinning of the capsule overlying the sensor and improved vascularity close to the sensor membrane surface.

**CONCLUSIONS** — These subcutaneous glucose sensor implants as studied in a dog model appear promising as a continuous and painless long-term method for monitoring glucose. A 90% response time of 4–10 min essentially provides a continuous real-time measurement of blood glucose. Furthermore, inherent in the sensor technology is important trend information: Is glucose stable, increasing, or decreasing, and, if decreasing, then how rapidly? Vibratory or auditory surveillance alarm functions can be added. By constructing the

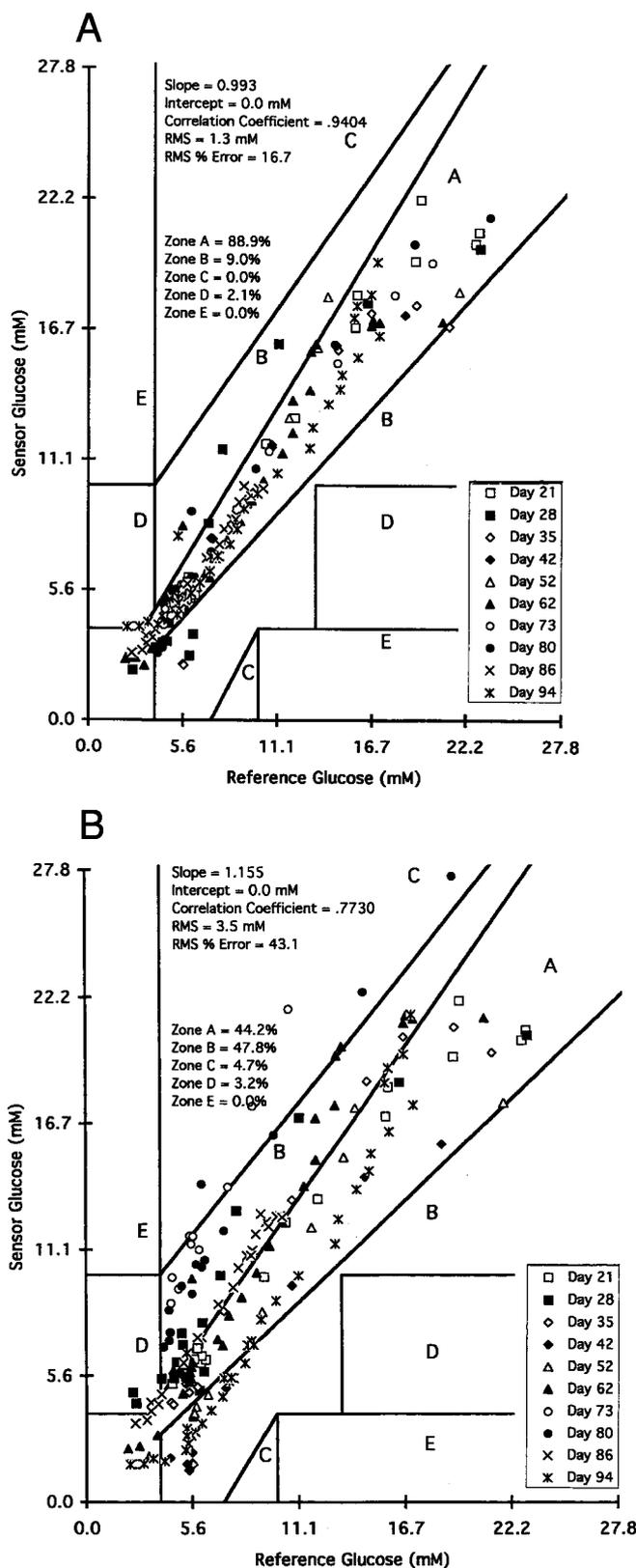


Figure 4—Summary plots of 10 in vivo sensor calibrations performed every 1–2 weeks from day 21 to 94. A: A retrospective calibration was used to optimize the sensor data to fit the reference blood glucose values for each day of the study. B: The same data as in C plotted to show retrospective calibration based on an average recalibration interval of once every 18 days. The data in this figure first appeared in a different format in Updike et al. (5). RMS, root mean square.

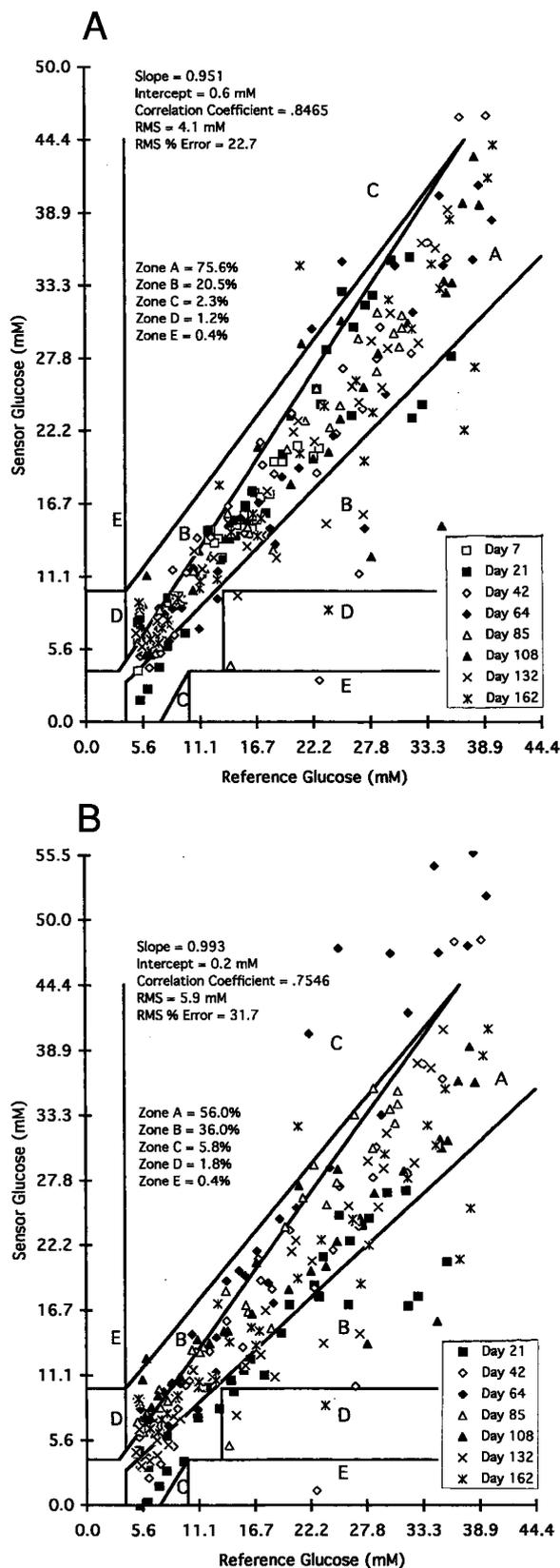


Figure 5—Summary plots of eight in vivo sensor glucose infusion studies performed every 2–4 weeks for 162 days ( $n = 255$  sensor vs. reference glucose determinations). A: Data plotted for each study by using retrospectively calculated calibration factors from the day of the study. B: Data plotted for days 21–162 by using a moving recalibration protocol with an average interval of 20 days. RMS, root mean square.

implant with microcircuitry, we believe this sensor can be miniaturized to about the size of a large jelly bean and could be implanted subcutaneously in the outpatient clinic under local anesthesia. Radiotelemetry readout devices can now be reduced to the size of a beeper worn on a belt or a wristwatch (13).

This type of glucose sensor has been extensively evaluated on the bench top (14), and the enzyme active membrane was mass manufactured by Markwell. The membranes used in the sensors whose in vivo data appear in Figs. 2–5 were actually manufactured 6 years ago. These membranes were stored at 6°C until final sensor assembly and in vitro testing on the bench top before implantation. Thus, evidence exists that membranes can be mass manufactured well in advance and cold stored until needed for final device assembly.

Multiple factors contribute to the stable and long-lived sensor performance we have demonstrated in the dog model. An enzyme electrode sensor is inherently simple because it is basically a diffusion limited device. Unlike blood glucose analysis in the clinical chemistry laboratory, an enzyme electrode sensor involves no dilution step, no moving parts, and no consumable reagents to be exogenously metered into the system. Proper choice of membrane polymer material essentially prevents oxygen limitation, and aggressive enzyme loading into the membrane provides a sensor lifetime of many months and possibly years (5,7). The addition of a bioprotective and angiogenesis layer over the tip of the sensor also enhances stability because these layers prevent biodegradation of the enzyme active membrane while encouraging vascularity at the capsule/sensor interface. With the right choice of biointerface materials, the FBC, at least in the dog model, can be coaxed into maintaining a long-term well-vascularized sterile constant temperature environment for the sensor.

All measurement devices must be periodically recalibrated to be reliable over the long term. Recalibration of a sensor implant is particularly challenging given its inaccessibility and the perceived inconvenience for the patient. Just how often this implantable sensor will require recalibration is not clear. Further development and optimization efforts may lead to still better stability and reliability.

Despite the progress shown, this sensor technology still suffers from multiple failure modes. The most common failures

are electronic and occur from loss of the quasi-hermetic packaging seal. This electronic leakage problem may result in changes in sensor baseline current that necessitate more frequent calibration or may escalate to total system failure if the power supply is interrupted. Failure can also occur from trauma, such as the dog rubbing or rolling on the implant wound, or from seroma formation around the sensor (typically within the first 3 weeks after implantation). The bioprotective layer may fail to prevent macrophages from reaching and degrading the enzyme active polyurethane membrane. This failure mode initially results in sensor calibration shifts and eventually in a total failure to respond to glucose. Finally, the FBC occasionally fails to provide adequately the oxygen and glucose needed to track blood glucose accurately. Such a failure occurs when the FBC/sensor interface becomes avascular, as seen with hematoxylin and eosin histology of the explanted tip of the sensor. We are optimistic that these problems can be resolved with future study.

In summary, stable clinically useful performance of these sensors was demonstrated as early as 7 days after implantation and for a lifetime of 5 months with response times of <10 min. This type of subcutaneous glucose sensor appears to be promising as a continuous and painless long-term method for monitoring blood glucose. The hope is that diabetic patients, even without addition of an insulin pump, when provided with painless comprehen-

sive glucose information will be able to modulate their diet, exercise, and insulin dosing to achieve safe tight glucose control.

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#### References

1. DCCT Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 329:977–986, 1993
2. U.K. Prospective Diabetic Study Group: Intensive blood-glucose control with sulphonylureas and insulin compared with conventional treatment and risk of complications in patients with type II diabetes. *Lancet* 352:837–853, 1998
3. Updike SJ, Hicks GP: The enzyme electrode. *Nature* 214:986–988, 1967
4. Gilligan BJ, Shults MC, Rhodes RK, Updike SJ: Evaluation of a subcutaneous glucose sensor out to 3 months in a dog model. *Diabetes Care* 17:882–887, 1994
5. Updike SJ, Shults MC, Rhodes RK: Principles of long-term fully implanted sensors with emphasis on radiotelemetric monitoring of blood glucose from inside a subcutaneous foreign body capsule (FBC). In *Biosensors in the Body*. Fraser DM, Ed. New York, Wiley, 1997, p. 117–137
6. Gerritsen M, Jansen JA, Kros A, Nolte R, Lutterman JA: Performance of subcutaneously implanted glucose sensors: a review. *J Invest Surg* 11:163–174, 1998
7. Rhodes RK, Shults MC, Updike SJ: Prediction of pocket-portable and implantable glucose enzyme electrode performance from combined species permeability and digital simulation analysis. *Anal Chem* 66:1520–1529, 1994
8. Updike SJ, Shults MC, Rhodes RK, Gilligan BJ, Luebow JO, von Heimburg D: Enzymatic glucose sensors: improved long-term performance in vitro and in vivo. *Trans Am Soc Artificial Intern Organs* 40:157–163, 1994
9. Stokes KB: Polyether polyurethanes: biostable or not. *J Biomater Appl* 3:228–259, 1988
10. Shults MC, Rhodes RK, Updike SJ, Gilligan BJ, Reining WN: A telemetry-instrumentation system for monitoring multiple subcutaneously implanted glucose sensors. *IEEE Trans Biomed Eng* 41:937–942, 1994
11. Baker DA, Gough DA: Dynamic concentration challenges for biosensor characterization. *Biosens Bioelectron* 8:433–441, 1993
12. Clarke WL, Cox D, Gonder-Frederick LA, Carter W, Pohl S: Evaluating clinical accuracy of systems for self-monitoring of blood glucose. *Diabetes Care* 10:622–628, 1987
13. Gaskill G, Grey K: High-Speed Subcarrier Data System for Radio Wristwatch. Beaverton, OR, SEIKO Telecommunications Systems (document no. 1004-DOC-0868), 1995
14. Updike SJ, Shults MC, Capelli CC, von Heimburg D, Rhodes RK, Joseph-Tipton N, Anderson B, Koch DD: Laboratory evaluation of new reusable blood glucose sensor. *Diabetes Care* 11:801–807, 1988