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Progress toward the Development of an Implantable Sensor for Glucose

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The development of an electrochemically based implantable sensor for glucose is described. The sensor is needle-shaped, about the size of a 28-gauge needle. It is flexible and must be implanted subcutaneously by using a 21-gauge catheter, which is then removed. When combined with a monitoring unit, this device, based on the glucose oxidase-catalyzed oxidation of glucose, reliably monitors glucose concentrations for as long as 10 days in rats. Various design considerations, including the decision to monitor the hydrogen peroxide produced in the enzymatic reaction, are discussed. Glucose constitutes the most important future target analyte for continuous monitoring, but the basic methodology developed for glucose could be applied to several other analytes such as lactate or ascorbate. The success in implementation of such a device depends on a reaction of the tissue surrounding the implant so as not to interfere with the proper functioning of the sensor. Histochemical evidence indicates that the tissue response leads to enhanced sensor performance.

Additional Keyphrases: *enzyme electrode · glucose oxidase · electrochemistry · subcutaneous sensor*

Determination of concentrations of glucose in blood has long been recognized as an important clinical diagnostic test for diabetes. Thirty years ago, Clark and Lyons (*1*) suggested coupling an enzymatic reaction to electrochemical monitoring in what was subsequently called an enzyme electrode. This device made possible the largely selective detection of glucose in both serum and whole blood, and commercially available clinical analyzers from Yellow Springs Instruments and Beckman Instruments, among others, have been available for some time. A significant breakthrough occurred in the early 1980s, when self-monitoring of blood glucose became possible through the development of dry chemical strips for use with a single drop of blood. The resulting reaction (again, typically involving the glucose oxidase-catalyzed oxidation of glucose) could be followed by monitoring the formation of product spectrophotometrically or electrochemically. More recently, the possibility of monitoring glucose by noninvasive near-infrared techniques has been proposed (*2, 3*). This procedure would eliminate the painful and annoying step of pricking the finger to obtain blood.

Although self-monitoring is considered a major advance in diabetes management, it is limited practically by the number of determinations a patient can reasonably be expected to make in a 24-h period. Most patients complain that making these measurements is boring and inconvenient, and they are not willing to take more than two or three measurements a day. Such a sampling frequency does not permit tight control of blood glycemia at values close to normal [(~1.00 g/L (5.5 mmol/L)] throughout the day. Normoglycemia research is hampered by the fact that induced hypoglycemia will occur, leading possibly to convulsions or loss of consciousness. This concern influences a strategy that, in the absence of reliable monitoring, results in control of glycemia at glucose concentrations well above normal. The interim results from the Diabetes Control and Complication Trial (DCCT) (*4*) suggest that chronically high concentrations of blood glucose are at least partly responsible for such subsequent complications as blindness or end-stage renal failure. Improved glycemic control has been demonstrated when monitoring is based on measurements of glycohemoglobin, but it is unlikely that the normal range can be reached without careful and continuous monitoring of glucose (*5*).

Accordingly, a strong argument can be made for the need of a continuous glucose monitoring system that is reliable, portable, and inconspicuous. Such a system would acquire time-dependent glycemia data that could be used by the physician as an aid in normalizing insulin therapy for a particular patient. It would also provide an alarm system to warn the patient when glucose values fall or are about to fall outside of the desired range. Ultimately, if the glucose sensor proved highly reliable, then perhaps its coupling to an insulin pump could be considered as an approach to the artificial pancreas.

Approaches to Glucose Sensing

In the early 1970s, attempts were made to develop systems for the automatic control of blood glucose concentrations, the best known of these being the Biostator® (Miles Labs., Elkhart, IN). This system requires external circulation of blood into a detection chamber, where the blood is diluted before being measured with an enzyme electrode. Insulin is then infused on the basis of the measured glucose concentrations. Unfortunately, this system requires catheterization, heparinization, and dilution of blood and is therefore useful only in a clinical setting. Further, the system is so bulky as to be

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useless for an ambulatory patient. Most important, however, is the lack of a sensor that can function reliably long term.

It is now well established that the concentration of blood glucose is the analyte of merit for diabetes management, therefore, direct monitoring of blood glucose should be the approach of choice. It is necessary, however, to balance the benefits of continuous monitoring against the risk of infection or blood clotting, which might result from implanting a sensor in the vascular bed. Thus, even though the feasibility of long-term monitoring has been demonstrated in dogs (*6*), it is unlikely that this approach would be readily accepted in humans. Two other sampling sites have been investigated in detail: the peritoneal cavity (*7, 8*) and subcutaneous tissue (*9–16*). Sensors implanted in the former site show rapid changes in glucose concentrations that correlate well with those in the blood (*17*). The utilization of this site would require surgical implantation and thus would dictate long-term sensor reliability, probably for several years or more. Not only would the sensor have to be implanted but also the monitoring unit.

Such considerations have led to the focus on the subcutaneous tissue as the site of choice. Several recent studies (*15, 16*) have established that the subcutaneous glucose concentrations are the same as the concentrations in blood, but the former lag behind rapid changes in blood glucose by ~5 min (*18*). The possibility of using subcutaneous monitoring as part of a closed-loop insulin delivery system has been demonstrated in diabetic dogs (*17*). Minimally invasive, this approach permits the sensor to be connected to a monitoring unit the size of a wristwatch. Several subcutaneous implantation sites are feasible, and such a sensor/monitoring unit combination would meet the requirements of a small unobtrusive continuous glucose monitoring system.

Fundamentals of Sensor Operation

By the early 1980s, the enzyme electrode had proven its capabilities in the in vitro measurement of blood or serum glucose, but in vivo measurements proved much more difficult. It was not possible to simply scale down a 0.2- to 2-cm (o.d.) sensor to a size that would be compatible with subcutaneous implantation. Nevertheless, Shichiri et al. (*9*) demonstrated that a needle-type sensor could be used for such measurements. For ease of implantation and for minimal pain associated with this process, the diameter of a practical sensor would have to be <0.5 mm, ideally ~0.35 mm (the size of a 28-gauge needle).

Practically all of the reported sensors with which continuous measurements have been made are electrochemically based and take advantage of the reaction of glucose with oxygen:

$$\text{Glucose} + O_2 \rightarrow \text{gluconic acid} + H_2O_2$$

This reaction can be monitored by following either the consumption of oxygen (Figure 1*A*) or the formation of hydrogen peroxide (Figure 1*B*) (*19*). The first method has the advantage that the oxygen can be electrochemically detected by simply putting a gas-permeable membrane in front of the electrode. The membrane excludes various electroactive endogenous molecules. However, because the rate of the reaction depends on the consumption of oxygen, it is necessary to measure the difference between the incoming oxygen flux and that which arrives at the electrode. Such an arrangement is intrinsically complicated, particularly for the very small sensor specified above. The second and most widely applied method involves detection of a product of the enzymatic reaction. This approach does not require a difference measurement but, because of the relatively high applied potential necessary to monitor hydrogen peroxide, interference from endogenous electroactive species such as urate and ascorbate must be considered.

A third method (Figure 1*C*) involves the use of mediators placed in the enzyme layer; these couple the oxidation of reduced enzyme to the electrode because it is not possible to carry out efficient and direct electrochemical oxidation of the enzyme. The mediator, in principle, replaces oxygen in the above reaction, functioning as the electron acceptor. The applied potential can often be much lower than in the second method (Figure 1*B*), so that interference from endogenous species can often be eliminated. However, because oxygen can freely diffuse throughout the enzyme layer and the mediator typically cannot, the ability of the mediator to compete with oxygen is often poor. This leads to a

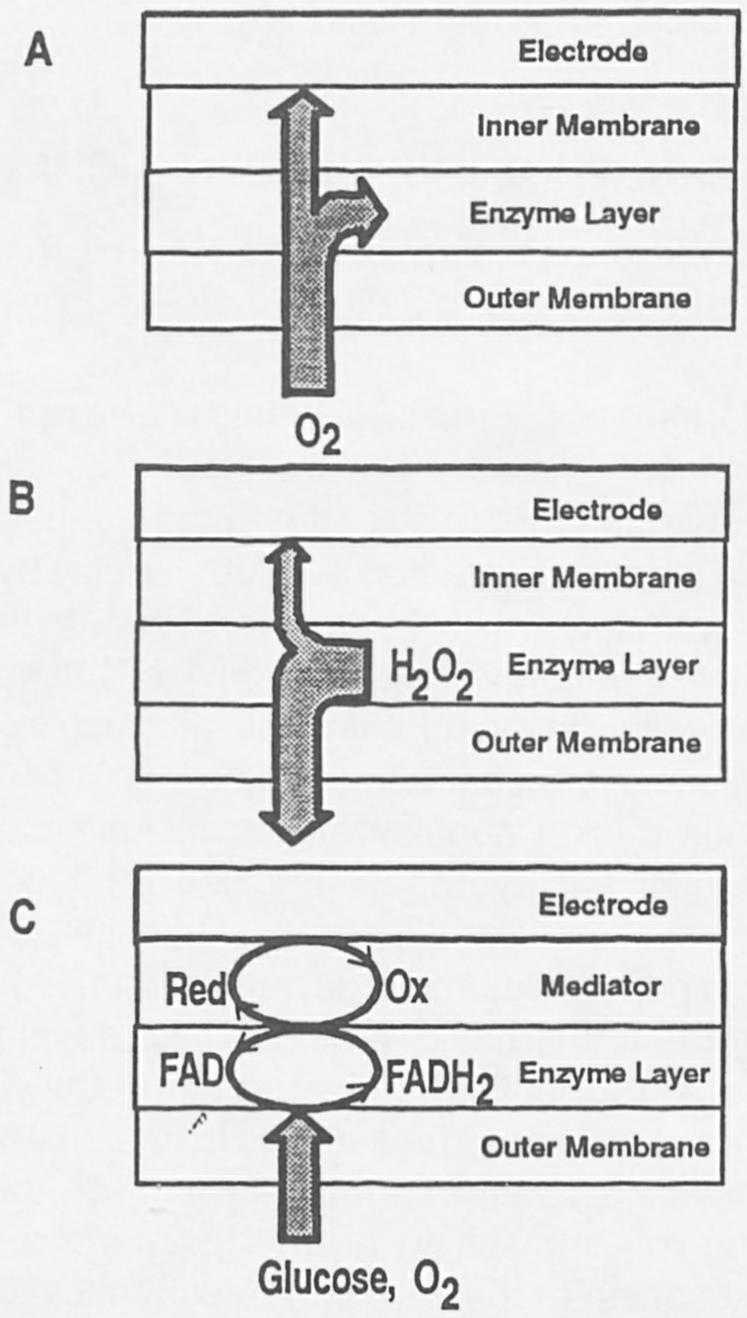

Fig. 1. Detection approaches for the glucose enzyme electrode: detection based on (*A*) oxygen; (*B*) peroxide; and (*C*) a mediator

Red and Ox correspond to the reduced and oxidized forms of the mediator, respectively; FAD and $FADH_2$ are the oxidized and reduced forms of the flavoenzyme prosthetic group. Adapted with permission from reference *19*

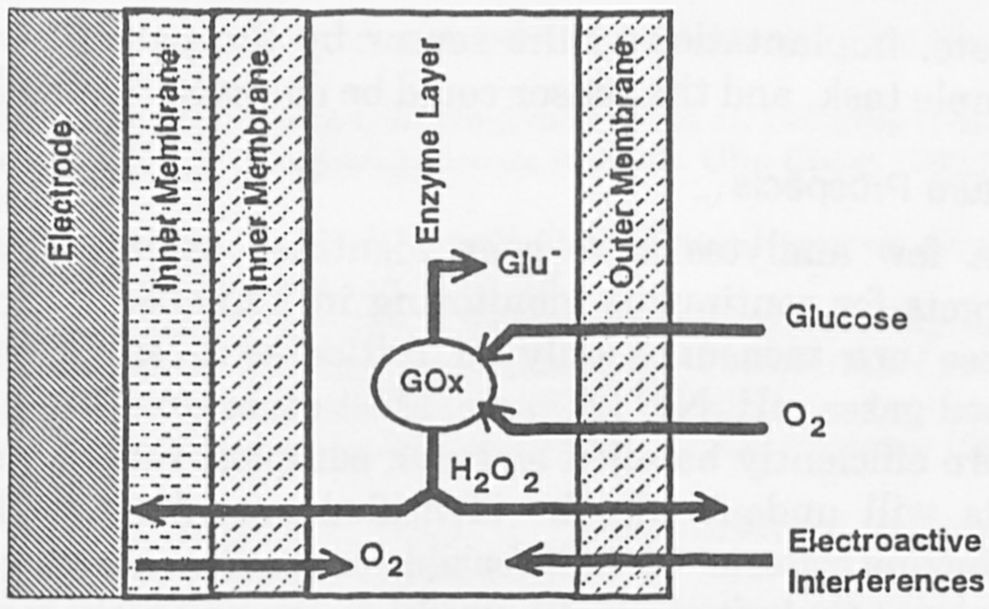

Fig. 2. Diagram of the multilayered sensing element, showing the fluxes of the various species present

GOx, glucose oxidase; glu⁻, reduced glucose

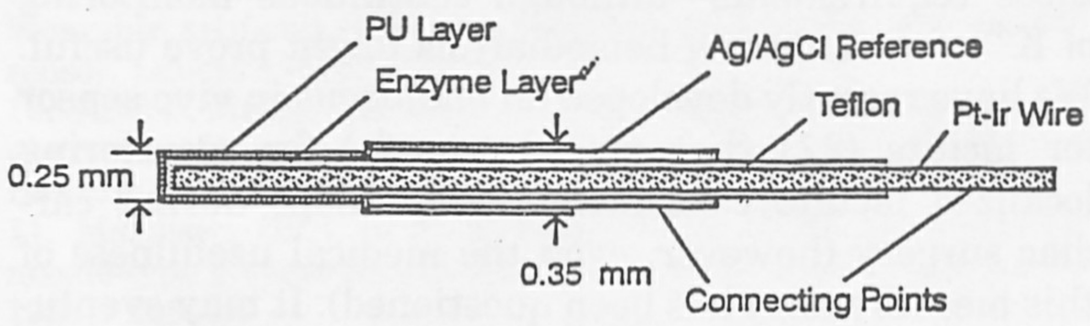

Fig. 3. Diagram of the implantable sensor

PU, polyurethane

parasitic influence of oxygen on the response. If the mediator is made more mobile, it escapes out of the enzyme layer into the biological medium. The use of mediators in sensors with good long-term stability will probably depend on the construction of very stable but very thin enzyme layers that contain fixed mediator sites. Work in this direction is proceeding in several laboratories.

The role of the outer membrane (Figure 2) is extremely important to sensor function. Proper adjustment of the permeability of this membrane to glucose renders the rate of the enzymatic reaction linearly dependent on the external glucose concentration up to ~15 mmol/L. In the case of our sensor (*20*), the use of polyurethane as the outer membrane significantly reduces the flux of glucose without materially affecting that of oxygen. The result is a sensor that depends on oxygen for its function but for which the response is nevertheless essentially oxygen independent. At 15 mmol/L glucose concentration, a properly prepared sensor will show <5% decrease in response as P_{O_2} passes from 120 to 1 kPa (*21*). At basal values, oxygen dependence is even less (the normal tissue content of oxygen is estimated at 2.7–3.3 kPa). The outer membrane also serves to protect the enzyme layer from the biological fluid, preventing the destruction of peroxide by, e.g., endogenous catalase. The outer membrane also interacts with the tissue, a process with an important influence on sensor function (discussed later). The entire sensor in its current form is shown in Figure 3. Its overall length is 4 cm, the diameter of the sensing element is 250 mm, and the overall diameter at the widest point (reference electrode) is 350 mm. This corresponds to the outside diameter of a 28-gauge needle. Because the sensor is extremely flexible, we can implant it by using a short (3 cm) stainless steel catheter (21 gauge), which is removed after the sensor is in place.

Because of the high applied potential necessary to detect peroxide (650 mV vs the Ag/AgCl reference electrode), the influence of endogenous electroactive species must be considered. Species such as urate and ascorbate are anionic and accordingly are retarded by the negatively charged inner membrane (cellulose acetate); therefore, they do not constitute a significant interference (*20*). Neutral molecules such as acetaminophen (paracetamol) are not retarded by the inner membrane layer; therefore, an additional polymer layer has been added. Preliminary in vivo studies support the effectiveness of this additional layer (unpublished results).

In Vivo Evaluation of Sensor Performance

A key element in evaluation in vivo is the establishment in situ of the relationship between the blood glucose concentration and the sensor response. This is accomplished by increasing the blood glucose concentration in normal animals such as dogs or rats by venous infusion (*22*) or by intraperitoneal injection (*23*), respectively, of glucose. The sensor response is measured at $t = 0$ (baseline) and again at a plateau of ≥5 min after the effect of the infusion is seen. At each of these points, the concentration of blood glucose is measured by using, e.g., the Beckman Analyzer or a self-monitoring strip. From this information, one can then establish the slope (sensitivity) and the intercept (background current) (*24*). This procedure provides a reliable calibration curve without having to assume that the in vitro and in vivo responses are the same. This procedure has been described elsewhere (*18, 24*).

Long-term sensor response has been evaluated in rats over the first 10 days after implantation (*23*). When the sensor is initially implanted, there is a period of 2–4 h during which the sensor response stabilizes. From day 1 to day 10, there is essentially no change in either the sensitivity or the apparent response time of the sensors (n = 7). We consider this an important finding and suggest that previous observations led to overly pessimistic conclusions. The small size of this sensor and the minimal tissue damage caused by its implantation may contribute to its superior performance.

We evaluated the performance of the sensors (*23*) after 10 days, using the error grid analysis method of Clarke et al. (*25*) shown in Figure 4 (n = 101). All sensor-measured values fell in zone *A* (clinically accurate), except for one value in zone *B* (clinically acceptable). The points below the correlation line are generally a manifestation of the lag time because the blood glucose concentrations increase faster than the subcutaneous ones. When glycemia is decreasing, the reverse is the case, and the sensor values lie above the correlation line. The lag time is due to the kinetics of glucose infusion and is not limited by the intrinsic sensor response time. These results show that, even if the effect of the lag time is ignored, there is no adverse effect on

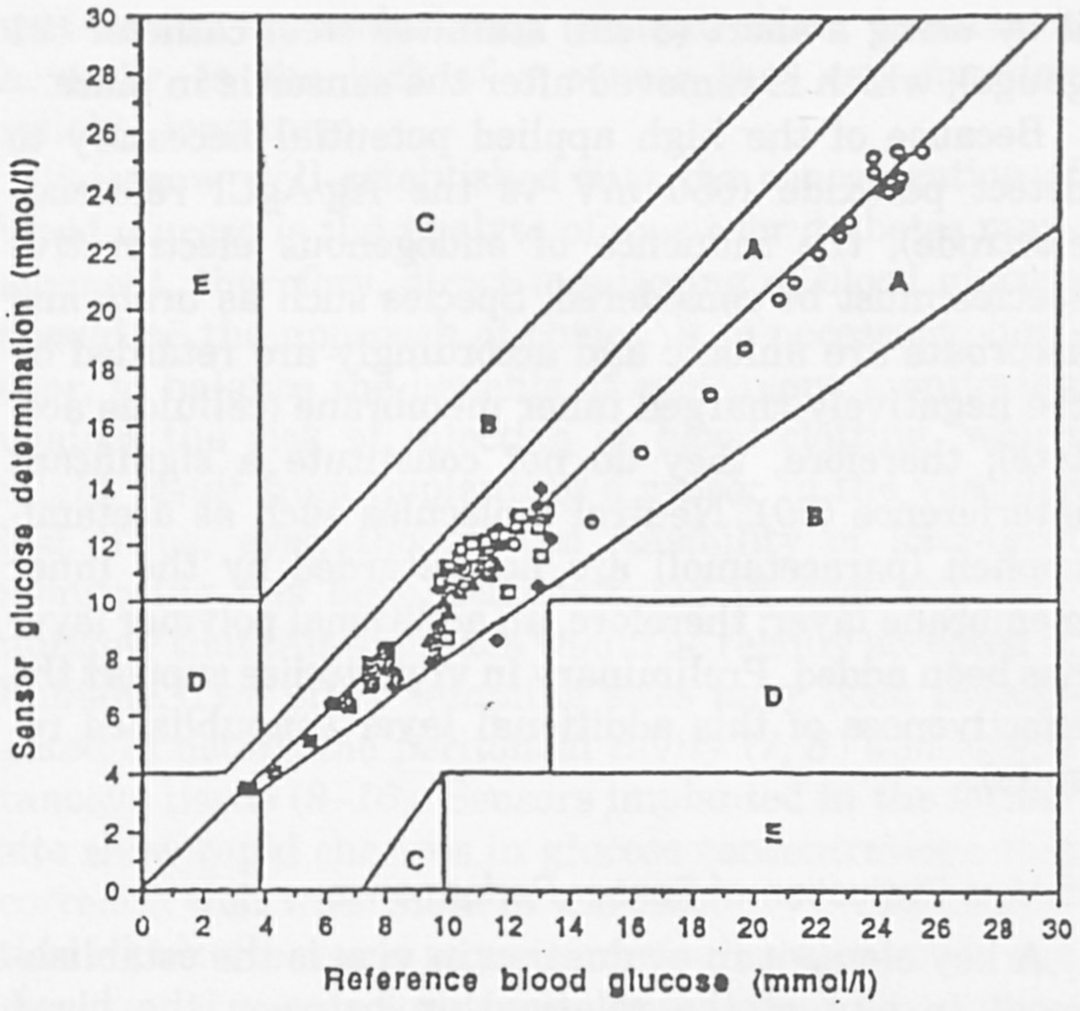

Fig. 4. Error grid analysis for estimating the accuracy of sensor-generated blood glucose values, 10 days after implantation

The plot is divided into five zones (*A–E*) on the basis of the clinical significance of the glycemia estimated by the sensor. *A:* estimation clinically accurate; *B:* acceptable estimation; *C:* inaccurate estimation; *D,E:* decision based on these estimations would lead to potentially hazardous decision (e.g., injecting insulin when glycemia is actually low). Reprinted from reference *23* with permission

the clinical conclusions derived from sensor measurements.

The implementation of the continuous monitoring system requires a unit that can maintain the appropriate applied potential between the indicating and reference electrodes while measuring the resulting current. The time-dependent fluctuations of this current are stored and transformed into an estimation of the blood glucose concentration. Such a system has been designed and constructed at the Ecole des Mines de Paris and is currently being evaluated. For practical application, the monitoring unit must be miniaturized to be compatible with its use as a wearable system.

Biocompatibility and Biostability

The subcutaneous implantation of the sensor would reasonably be expected to produce a tissue response. Indeed, histological examination of the implant after 3 days showed the formation of fibrovascular tissue around the site of implantation, accompanied by a few macrophages, plasma cells, and polymorphonuclear cells (*23*). There was also evidence of neovascularization, which might serve to improve delivery of glucose and oxygen to the sensor surface. Previous studies have shown that the sensor is not toxic (*26*). Thus the sensor is not inert, but elicits a tissue response that actually improves sensor performance by regenerating the oxygen and glucose delivery system.

It is not yet known how long such sensors might be implanted in humans, the clinical studies of this having just begun. Experience with transdermal implantation of catheters suggests that 5–7 days would be the maximum, provided that care is taken to keep the penetration region sterile. This period of time should be adequate: implantation of the sensor by the patient is a simple task, and the sensor could be changed regularly.

Future Prospects

A few analytes have been identified as important targets for continuous monitoring in humans. Most of these are measured only in critical-care situations: blood gases, pH, Na^+, K^+, etc. Most other analytes are more efficiently handled as batch samples. Future targets will undoubtedly be identified according to the following criteria: (*a*) Is it feasible to monitor the target analyte? (*b*) Is it clinically useful to continuously measure this analyte? (*c*) Is there a sufficiently large market for a monitoring system to justify the necessary research and development? So far, only glucose meets these requirements—although continuous monitoring of K^+ or urea during hemodialysis might prove useful. We have recently developed an analogous in vivo sensor for lactate (*27*) that may be useful for monitoring localized lactate concentrations—perhaps during cardiac surgery (however, even the medical usefulness of this measurement has been questioned). It may eventually be possible to monitor therapeutic drugs that have a narrow therapeutic range, especially those that might be infused at a controlled rate. If patients are going to use these monitoring systems themselves, the systems must be simple and inexpensive. More probable is the development of in vivo enzyme electrode sensors for neuroactive substances, especially those that are not intrinsically electroactive (*28*). Such applications will probably be confined to the neuroscience research laboratory.

A fundamental question is whether the sensor, which must necessarily be quite small, is properly sampling the site of implantation and also whether this site is indicative of the region of interest. Sensors based on amperometric detection (such as glucose) are typically coupled to an enzyme that catalyzes an oxidation–reduction reaction. The analyte provides one of the necessary redox couples; the other, which will be consumed in the reaction, must also be provided. This is the reason why oxidases have been so widely used: oxygen is almost always present. A very large group of enzymes use the NAD^+/NADH couple as a coenzyme; however, this couple is used very little in enzyme electrode applications because the coenzyme, which functions as a mediator, has many of the limitations of the mediator described above.

Clearly, if reliable, low-cost, noninvasive sensors can be developed, they will be the approach of choice. In the interim, minimally invasive devices may fill a critical gap.

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