CHAPTER 1

INTRODUCTION TO THE GLUCOSE SENSING PROBLEM

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1.1 A SHORT HISTORY OF DIABETES AND GLUCOSE MEASUREMENT

Although the subject of some controversy, the Ebers papyrus (1550 BC) appears to be the earliest, largest, and most comprehensive reference to diabetes and describes one of the principal symptoms of the disease, excessive urination. Other scholars consider the discussion sufficiently vague that it may be regarded as a kidney disorder. In the second century AD, however, the condition was described in more detail by Areteus and the focus was on excessive urination, unquenchable thirst, and degradation of tissue. The name diabetes, taken from the Greek, siphon, was adopted, because fluid does not remain in the body.

There are various references to this condition also noting urine that is sweet, owing to the discharge of glucose when blood levels rise above a threshold for a particular patient. In China, the brilliant physician Zhang Zhongjing noted around AD 200 the ''malady of thirst.''[1]

In the second millennium AD, diabetes was diagnosed by ''water tasters'' who tasted the urine of patients to establish that it was sweet tasting. This resulted in a second general property, sweetness, and hence the term *mellitus*, coming from the Latin word for honey. Mathew Dobson, a British physician and chemist, suggested in 1766 that the sweetness in both urine and serum was due to sugar.[1] Throughout the seventeenth and eighteenth centuries, the sweetness of urine was used as an indicator of diabetes. It was observed that diabetes was fatal in less than 5 weeks in some (type 1) and a chronic condition (type 2) for others. It was also observed that urine glucose was reduced as the result of a high-protein, high-fat diet, whereas starchy food produced high sugar levels in the urine and blood. In the early to mid-nineteenth century, attention turned to consideration of diabetes as a metabolic disease and to reach such conclusions it was necessary to develop analytical tools that would enable glucose levels to be reliably monitored.

By the turn of the twentieth century, it was realized that diabetes was associated with the pancreas. In 1921, Banting and Best successfully isolated insulin from dog pancreas, and this was followed quickly by tests in humans. Eli Lilly began production of insulin in 1923.

1.1.1 Chemical Methods for Glucose Measurement

The advent of analytical methodology for glucose measurement began to show its influence through the work of Bernard, Bouchardat, and chemists Priestley, Lavoisier, Chevreul, and Wöhler.[1] They focused on chemical transformations linked to metabolism. As Claude Bernard, the eminent French physiologist, noted in his lectures at the College de France in the 1870s,[2] there were three methods commonly used at that time to detect glucose: polarimetry (rotation of polarized light), reduction of Cu(II) to Cu(I) by reducing sugars (Barreswill/Fehling), and the evolution of CO_2 resulting from the fermentation of a glucose-containing solution. These methods were first applied to the determination of glucose in urine, especially the work of Bouchardat, who is recognized as the first clinician to suggest regular monitoring of glucosuria (urine glucose) and also to specifically suggest that this should be the patient's

responsibility.[3] Using the reducing sugar method (Fehling's solution) to measure glucose in blood (25 g of blood), Bernard was able to establish that glucose could be generated endogenously from glycogen and detected through its appearance in the blood that was not attributable to carbohydrate ingestion (gluconeogenesis). To make this discovery, it was necessary to develop an analytical method for the analysis of blood samples. He clearly realized and pointed out to his students[2] that the reduction of Cu(II) is not specific for glucose. In spite of the limitation of this method, it persisted for almost 100 years as a dominant glucose analysis approach along with the closely related Benedict's solution. In 1941, Miles Laboratories (now Bayer) developed the Clinitest®, essentially the Benedict's reagent in tablet form, which when added to the sample gave rise to an exothermic reaction necessary to facilitate the Cu(II) reduction. In 1956, a dip-and-read test for glucose in urine was developed by Bayer (Clinistix®). This represented a significant departure from previous technology since it employed glucose oxidase and peroxidase so that the peroxide formed from the reduction of oxygen could react with *o*-toluidine to give rise to a color development. The color was then compared with a color scale designated negative, light, medium, or dark. The test was useful for diabetic patients in determining whether glucose levels were above the renal threshold, but when the glucose levels were normal, little glucose was found in urine. In 1964, a test strip, based on the same technology (Dextrostix®), was developed for the measurement of glucose in blood by Anton (Tom) Clemens. Another related and significant development (1979) was the availability of a lancing system that simplified the blood sampling process, the Ames Autolet®, a fingerprick device.

1.1.2 Instrumental Readout for Glucose Strip Measurements

Rather than relying on comparison with a color scale, a reflectance meter was developed to read the Dextrostix strip called the Ames Reflectance Meter, primarily meant for use in doctors' offices. It was rather expensive ($495) but was used by a few patients. A version for patients that also provided a memory for results was introduced as the Glucometer® in 1985. A number of companies continued to produce reflectance meters, but in 1985 an electrochemically based test strip was described by Cass and coworkers.[4] In 1987, the Medisense ExacTech® sensor was launched. This device incorporated the use of an exogenous mediator as the electron acceptor, coupling the oxidation of reduced enzyme to the electrode. Since that time, the number of companies producing test strips has proliferated to more than 30. Both glucose oxidase and glucose dehydrogenase are employed. Electrochemical detection is the dominant technology for strips and improvements have come in the form of less blood (now around 0.2 μL) and less painful sampling, faster measurements, and data systems to help with diabetes management.

1.1.3 Glucose Biosensors for Clinical Applications

In the mid-1950s, Leland Clark developed an electrochemical method for oxygen measurements in biological fluids and made the discovery that if the Pt electrode used for detection could be separated from the biological medium by a gas permeable

membrane, reliable measurements were possible. This approach was extended by immobilizing glucose oxidase on a membrane and measuring the reduction in oxygen levels as a result of the enzymatic reaction.[5] A miniaturized form of this sensor was proposed and referred to as the enzyme electrode.[6] In 1974, the Model 23 Yellow Springs Instruments glucose analyzer appeared in the market and is still used for clinical glucose measurements. The concept of an electrochemical biosensor has evolved into many different devices capable of continuous glucose measurements as well as a number of other analytes. Clemens at Miles was also responsible for developing a closed-loop system called the Biostator® that sensed blood glucose levels through an extracorporeal shunt and delivered insulin and/or glucose according to a control algorithm. The system was too massive and insufficiently reliable to serve ambulatory patients, but it did focus attention on glycemic control algorithms and on the importance of normoglycemia.[7–9]

1.1.4 Continuous Glucose Monitoring Systems (Electrochemical)

A report by Shichiri in 1982 involving the use of a subcutaneously implanted needle-type sensor in a pancreatectomized dog launched the quest to develop wearable systems for continuous glucose monitoring.[10] At the Central Diabetes Institute, Karlsburg, GDR, Fischer and coworkers examined subcutaneous monitoring in significant detail and demonstrated automated feedback control of subcutaneous glucose.[11–13] The possibility of sensor indwelling in the vascular bed was demonstrated in dogs by Gough and coworkers for sensors that survived for several months. Sensor failure was generally due to a failure of the electronics and not the sensor itself.[14] This technology was licensed to Medical Research Group, Inc. that later merged with Medtronic Minimed. A prototype intravenous catheter-type glucose sensor was developed. Clinical trials in type 1 diabetic patients were initiated in 2000 in France and the United States. Some sensors remained functional for up to a year based on a special nonlinear calibration algorithm. Beyond that, the longevity of the sensor was mainly compromised by a gradual loss of enzyme activity.

In 1993, the report of a 10-year study by the Diabetes Control and Complications Trial (DCCT) Study Group concluded that intensive insulin therapy (multiple injections of insulin daily and control of glycemia closer to the normal level) resulted in a 30–70% reduction in the complications of type 1 diabetes.[15] Coupled to this, however, was an increase of 300% in the incidence of hypoglycemia. Since this is a major concern for patients and their physicians, it was evident that if tighter control could be linked with hypoglycemia avoidance, the significant benefits could be realized. More recently, it has been concluded that the history of HbA1c measurements in patients does not adequately explain the risk for development of chronic complications. Glycemic excursions or variability may be as important as chronic hyperglycemia in the development of chronic complications.[16] This lends further support for the need of continuous monitoring systems.

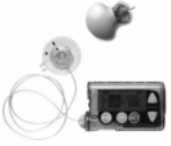
In 1993, we reported on a study of nine normal subjects using a wearable continuous monitoring system.[17] This was the first application of error grid analysis

(EGA)[18] to demonstrate continuous monitoring sensor performance (see below). More than 99% of all values were demonstrated to fall in the A and B zones. By the end of the 1990s and the beginning of the twenty-first century, four systems have been approved by the FDA: CGMS Gold®/Guardian RT® (Minimed/Medtronic),[19,20] GlucoWatch Biographer (Cygnus/Animas),[21–23] DexCom STS (DexCom),[24] and FreeStyle Navigator® (TheraSense/Abbott).[25,26] All of these systems still exhibit instability over the approved 3–7-day period of implantation. Patients are accordingly advised to make as many as four fingerstick measurements per day. Each calibration is usually assumed to be valid for no more than 12 h. The time-dependent results are now generally available to the patient, but they are advised to use the continuous monitoring systems to detect ''trends,'' while using the more reliable ''fingerstick'' systems to confirm results. The latest versions of four such monitoring systems are summarized in Table 1.1. Two systems have been developed in Europe that employ microdialysis sampling followed by detection with a conventional enzyme-based glucose sensor GlucoDay (Menarini Diagnostics)[20,27] and the Roche SCGM1.[28] These systems will be discussed in more detail elsewhere in this book. Despite the limitations of the continuous monitoring systems, valuable information has been obtained relating to insulin therapy. These include understanding the incidence of nocturnal hypoglycemia and arriving more quickly to improved metabolic control of patients.

1.1.5 Glucose Monitoring Systems (Nonelectrochemical)

All the above systems employ glucose oxidase and some electrochemical method for assessing the rate of the enzyme-catalyzed oxidation of glucose. There are two fundamental disadvantages of this approach: the sensing element must be in direct contact with biological fluid and the process of measurement requires the consumption of glucose. For these reasons, several alternative approaches have been developed that will be described in more detail elsewhere in this book. In general, however, they fall into two categories: first, spectroscopic measurements (optical rotation, near-infrared, Raman) for which a specific molecular signature has been identified, and second, measurements of glucose binding to specific agents where spectroscopic changes permit the binding process to be followed (fluorescent or other optical changes associated with glucose binding to boronic acid derivatives or binding to lectins such as concanavalin A).[29,30] Such methods have the advantage that they do not consume glucose in the course of making the measurement and this may prove to be an advantage in situations where the total amount of available glucose is limited. These methods are not automatically ''noninvasive'' as they may involve an implant that is externally interrogated or a device that enables interrogation of tear fluid via a contact lens.[31] Implanted devices can suffer from many of the same problems as the electrochemically based devices. Detection by near-infrared spectroscopy is probably the most advanced of the spectroscopic approaches and can, at least in principle, function as a truly noninvasive device.[32] Recently, a noninvasive system based on photoacoustic technology has been proposed (Aprise™, Glucon).[33]

TABLE 1.1 Continuous Glucose Sensors

Feature	Abbott FreeStyle Navigator®	MiniMed Paradigm® REAL-Time System	MiniMed Guardian® REAL-Time System	DexCom™ Seven™
Photos	 Photo from Abbott	 MiniMed Paradigm REAL-Time with new, smaller Mini-Link™ Transmitter; photo from Medtronic MiniMed	 Guardian REAL-Time System with new, smaller MiniLink™ Transmitter; photo from Medtronic MiniMed	 Photo from DexCom
FDA approval	March 13, 2008 for adults 18 +	Children 7–17 and adults 18 +	Children 7–17 and adults 18 +	March 2006 for adults 18 +
Accuracy	Error grid: 96.8–98.4% A + B	Consensus error grid: 98.9% A + B; MARD[a] (mean): 19.7%; MARD (median): 15.6%	Consensus error grid: 98.9% A + B; MARD (mean): 19.7%; MARD; (median): 15.6%	Clark error grid: 97% A B; MARD (mean): 15.7%; MARD (median): 11.4%
	GlucoWatch Biographer data for comparison: MARD: 17–21%; Clarke error grid A + B: 94%; Clarke error grid A: 60%			
Sensor life	Five-day wear indication	FDA approved for 72 h; users report longer wear times	FDA approved for 72 h; users report longer wear times	FDA approved for 7 days
Length of sensor probe	6 mm	0.5 in.	0.5 in.	13 mm

Gauge of sensor probe		23	23	26
Angle of sensor insertion	90°	45°	45°	45°
Insertion device available	Each sensor has a disposable inserter	Sen-serter®, manual insertion also possible	Sen-serter®, manual insertion also possible	DexCom SEVEN Applicator
Monitor size	3 in. × 2.5 in.	Displays on insulin pump, no separate monitor	3 in. × 2 in.	3 in. × 2.5 in.
Startup initialization time	10 h	2 h	2 h	2 h
Calibration	Calibrate at 10, 12, 24, and 72 h after insertion with no further calibration for the final 2 days of the 5-day wear	First calibration is 2 h after insertion. Second calibration within next 6 h after first, and then every 12 h. Will alarm if calibration value not entered	First calibration is 2 h after insertion. Second calibration within next 6 h after first, and then every 12 h. Will alarm if calibration value not entered	Must calibrate with One Touch Ultra—cannot be entered manually. Calibrate every 12 h, first calibration must have 2 done within 30 min of each other
Transmitter/sensor or body surface size	2 in. × 1 in. (combined)	Sensor the size of a nickel. Transmitter is 1.4 in. × 1.1 in. × 0.3 in. and attaches to the sensor	Sensor the size of a nickel. Transmitter is 1.4 in. × 1.1 in. × 0.3 in. and attaches to the sensor	2.5 in. (both combined)
Alarms on user—set low and high thresholds	Yes	Yes	Yes	Yes

Note: In clinical trials, some people never respond to alarms at night regardless of the volume. An alarming device (receiver or pump) that is under covers, a pillow, or underneath a body is almost impossible to hear. If you are considering a continuous glucose sensor, be sure to investigate the device's alarms to see if they will meet your needs

(continued)

TABLE 1.1 (*Continued*)

Feature	Abbott FreeStyle Navigator®	MiniMed Paradigm® REAL-Time System	MiniMed Guardian® REAL-Time System	DexCom™ Seven™
Displays glucose numbers	Every 1 min	Every 5 min	Every 5 min	Every 5 min
Displays directional trends	Yes, always has directional and rate of change arrow. Can view 2, 4, 6, 12, or 24 h glucose graph. Can go back 28 days	Yes, arrows that display how fast and in what direction, and 3 and 24 h graphs	Yes, arrows that display how fast and in what direction, and 3, 6, 12, and 24 h graphs	Yes, can display a 1, 3, or 9 h glucose graph
Displays rate of change	Yes, sideways arrow means dropping at less than 1 mg/dL/min. Up or down arrow means raising/dropping at over 2 mg/dL/min. 45° arrow means dropping/raising between 1 and 2 mg/dL/min	Yes, single and double arrows up or down communicate how fast glucose levels are falling or rising	Yes, single and double arrows up or down communicate how fast glucose levels are falling or rising	No
Predictive alarming	Yes, alarm on 10, 20, or 30 min before it thinks that you will hit that number based on the current trend. It estimates a future number by using algorithms and vector technology. (One parent has her child's alarm set at 20 min notice for highs and 30 min notice for lows)	No	Yes. Predictive alerts can be set to warn you 5, 10, 15, 20, 25, or 30 min before glucose limits have been reached. Rate of change alerts can be set to warn you when glucose levels are changing between 1.1 and 5 mg/dL/min, in 0.1 increments	No

Range of monitor to transmitter (factory stated)	10 ft (reports of significantly greater distance)	6 ft	6 ft	5 ft
Sensor storage: refrigerated or room temperature	Room temperature; 4 months life	Storage between 36 and 80°F without the need for refrigeration; 6 months life	Storage between 36 and 80°F without the need for refrigeration; 6 months life	Room temperature; 4 months life
Built-in BG monitor	Built-in Freestyle monitor	BD meter RF to pump or manually enter with other meters. With BD leaving market, future plans unknown	No, can use any meter and manually enter	Must calibrate with One Touch Ultra
Computer software	Freestyle CoPilot	Carelink™ Personal Software	Carelink™ Personal Software	DexCom DM Consumer Data Manager ($79)
Developing technology	Working on communications with a pump	Closed-loop sensor and pump in clinical trials (Yale, elsewhere)		Long-term (about 1 year/outpatient procedure) implantable sensor

From Children with Diabetes (www.childrenwithdiabetes.com). Used with permission.

[a] MARD is defined in equation (1.5).

1.2 SENSOR DESIGN

1.2.1 The Reactions

The discussion of sensors, especially those used for continuous monitoring, will be based on biosensors using glucose oxidase with electrochemical detection. The sensors generally adhere to the following sequence of reactions of enzyme, E, and mediator, M:

$$E_{ox} + \text{glucose} \rightarrow E_{red} + \text{gluconic acid} \quad (1.1)$$

$$E_{red} + O_2 \rightarrow E_{ox} + H_2O_2 \quad (1.2)$$

$$E_{red} + M_{ox} \rightarrow E_{ox} + M_{red} \quad (1.2')$$

An exogenous mediator such as ferrocene or Os(III) may be employed to accept electrons from the reduced enzyme, but it must be realized that reaction (1.2) can still take place because oxygen will be present in any case. Oxygen can diffuse freely within the reaction layer but the mediator cannot because it will be anchored to prevent leaching out of the sensor. The enzymatic reaction obeys Michaelis–Menten kinetics according to what is called a ''ping-pong'' reaction, the sequence of reactions (1.1) and (1.2)/(1.2′). The objective in sensor design is to make reaction (1.1) the rate-determining step, meaning that its rate is proportional to the concentration of glucose. For this to be the case over a range of glucose concentrations between 2 and 20 mM, reaction (1.2)/(1.2′) must be very rapid with respect to reaction (1.1). Otherwise the sensor response becomes dependent on O_2 or mediator concentration and will, at higher concentrations, yield a response independent of glucose. Thus, if the conditions are properly arranged, the rate of reaction (1.1) can be determined by measuring the rate of disappearance of O_2, the formation of H_2O_2, or the formation of M_{red}. The concentrations of the electroactive products/reactants of reaction (1.2)/(1.2′) will be monitored as

$$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2 \quad (1.3)$$

$$H_2O_2 \rightarrow 2H^+ + 2e^- + O_2 \quad (1.4)$$

$$M_{red} \rightarrow M_{ox} + 2e^- \quad (1.4')$$

The mediator is normally chosen such that its oxidation (reaction (1.4′)) occurs at a potential lower than that for direct electrochemical oxidation of ascorbate and urate, two endogenous electroactive species. If reaction (1.4) is used as the basis for glucose monitoring, then some measure (usually a permselective membrane) must be taken to prevent endogenous interferences from reaching the electrode. Mediators can react directly with endogenous electroactive species. If reaction (1.4′) is used as a measure of the rate of the enzymatic reaction and oxygen is not excluded, then reaction (1.2) will have a parasitic effect on the response, leading to low results.[34] Heller and coworkers developed polymeric matrices in which the mediator could be immobilized and therefore would not diffuse out of the reaction layer. These are referred to as ''wired'' enzyme systems as they connect the redox chemistry of the enzyme to the electrode.[35,36] In some systems, the rate of the reaction is, in effect, measured by determining the charge necessary to oxidize the product of the

two-reaction sequence (M_{red} or H_2O_2). This requires that the reaction is carried out in a fixed volume of sample for a defined time.

1.2.2 Control of Mass Transfer Using Membranes

The proper operation of a sensor based on the detection of peroxide (reaction (1.4)) can be controlled by the use of permselective membranes as shown in Figure 1.1. A membrane is located proximal to the electrode having the property of being permeable to peroxide, but not to endogenous electroactive species. Next is the enzyme layer, followed by an external membrane. In order to meet the condition noted above, this membrane is highly permeable to oxygen, but the permeability of glucose is significantly restricted. This property serves several useful purposes. First, as a consequence of reactions (1.1)/(1.2) above, glucose and oxygen should react in 1:1 stoichiometry, even though the tissue oxygen concentration is typically an order of magnitude lower than glucose. The membrane, in effect, creates a situation in the enzyme layer where oxygen is actually in excess. Second, the activity of the enzyme is made sufficiently high that glucose is immediately oxidized on its arrival in the enzyme layer. The rate of arrival (flux) and therefore the rate of the enzymatic reaction is defined by the concentration gradient between the outside and the inside of the outer membrane. Thus, as long as there is sufficient enzyme activity present, its exact activity does not matter and the sensor response will be limited by mass transfer and not by the rate of the enzymatic reaction. This is quite important because the activation energy for mass transfer is 3–4 kcal/mol as opposed to an enzyme-catalyzed reaction, which is ~11.8 kcal/mol at 300K.[37,38] This leads to temperature coefficients of 1.6–2.2%/°C and 7%/°C, respectively. The arrangement also accommodates small losses of enzyme activity without affecting the sensor sensitivity.

For blood glucose monitoring, it will generally be necessary to obtain linear sensor response in the range of 2–20 mM. In an air-saturated solution, the Michaelis constant (K_m) for glucose will be 5–6 mM. This corresponds to the substrate concentration yielding half the maximum reaction rate velocity. The maximum rate would occur at a concentration of about 15 mM, leading to mostly nonlinear

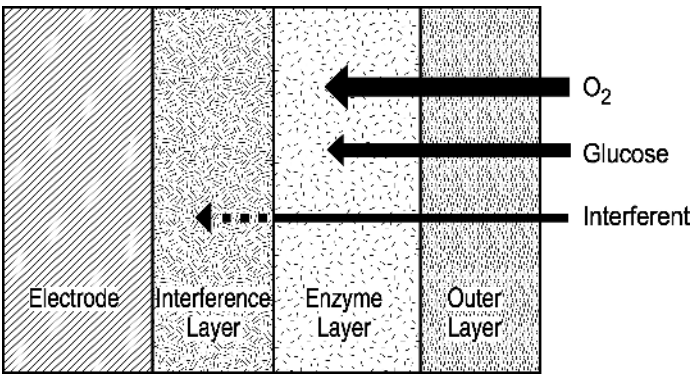


Figure 1.1 Multilayer sensor structure showing control of oxygen, glucose, and electroactive interferences.

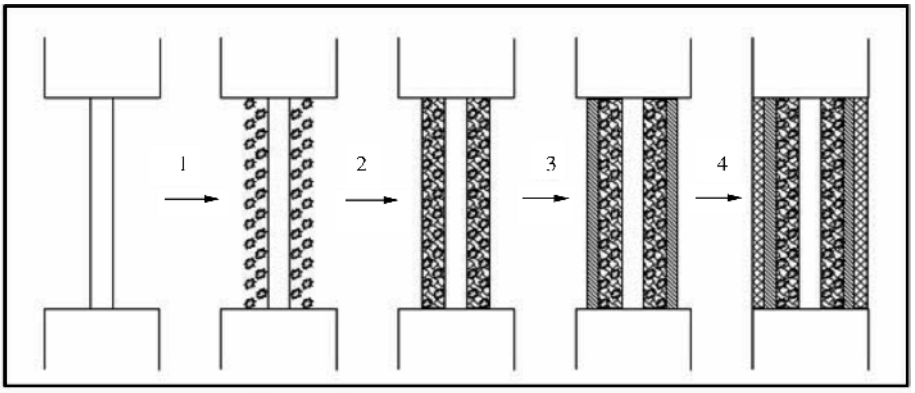


Figure 1.2 Electrochemical deposition of glucose oxidase (GOx) followed by electropolymerization of the polyphenol interference layer. Reprinted with permission from Ref. 40. Copyright 2002 American Chemical Society.

behavior starting at 10–11 mM. Thus, to obtain linear response over the required linear range, the K_m has to be much larger. There are two ways to do this. The first involves the lowering of the effective glucose concentration as noted above. The second approach involves increasing the rate of reaction (1.2)/(1.2′). Heller and coworkers have increased the effective K_m for the reaction to at least 40 mM using these two approaches.[39]

It is possible to combine the enzyme layer with the interference elimination layer through the electrochemical deposition of these layers in sequence. The enzyme layer is deposited first and the interference layer electropolymerized through it.[40,41] This is shown in Figure 1.2. The enzyme is first deposited on the electrode, and a key condition for the production of a smooth, compact enzyme layer is the presence of a detergent above its critical micelle concentration. Dynamic light scattering experiments show that the resulting micelle is approximately of the same diameter as glucose oxidase. The exact significance of this is unclear, but the detergent does prevent enzyme clumping during the deposition process. It is then possible to electropolymerize an interference reducing layer through the oxidation of phenol. The thickness of this layer can be conveniently controlled by controlling the electrodeposition conditions.

1.2.3 The Electrochemical Cell

The oxidation of hydrogen peroxide is actually quite complicated and depends upon the formation of a metal oxide on the electrode surface.[42–44] Consequently, there is no

advantage to changing the applied potential, and a DC applied potential of 0.65 V versus a AgCl/Ag reference electrode will suffice. An often overlooked problem is the stability of the reference electrode. There are a number of species present in biological fluids that can dissolve the AgCl deposit off the electrode by forming soluble Ag(I) complexes. This could include endogenous amines including peptides and proteins. The surface must therefore be protected with a membrane that is Cl^- permeable. It is not necessary to use a conventional three-electrode system since the currents typically encountered are in the nanoampere range or less.

1.3 DATA ACQUISITION AND PROCESSING

1.3.1 Acquisition and Readout Device

In general, data acquisition involves digitization of analog signals by a microprocessor. The procedure of data acquisition itself is fairly straightforward. Data processing, however, is where most effort is required. Each device developer has spent substantial resources in dealing with abnormalities representing real-life challenges. This is the fundamental difference between data from a traditional lab instrument and a patient-wearable device. The wearable device may experience a strong electromagnetic field, a shower, moving from a warm to a cold environment, or impact on the monitoring unit or sensor that generates spurious signals. The device must be able to identify such results and handle them according to a predefined methodology. Therefore, error handling becomes an important task. Being able to identify such errors and correctly classify them is a continuing challenge to monitoring system developers. In some cases, sensor drift and calibration instability can be handled retrospectively, but this is not of much use for a real-time monitoring system.

Regardless of the detection mechanism, a typical data processing algorithm must include the following steps (with the sequence interchangeable):

- Error identification and handling
- Signal separation from background
- Filtering and noise reduction
- Signal drift adjustment (if any)
- Conversion of signal to the desired form and unit (glucose in mM or mg/dL)
- Further filtering and restrictions based on known physiological or medical facts
- Calculations of parameters of clinical significance: average, trend, rate of change, and so on
- Database management

The physical forms of readout and alarms depend on the status of the instrumentation. A handheld device with LCD display and input buttons/pads is adopted by most manufacturers. An audio alarm is also available. An on-screen menu-driven method is used for setting up user features such as display content and styles. A historical glucose graph is also available.

1.3.2 Management Software

The most desired outcome of a glucose sensor system is to prevent the occurrence of hyper- and hypoglycemia or, at least, reduce the severity of hypoglycemia. For a stand-alone monitor system, all it needs to do is to sound an alarm accurately and in a timely fashion. This, in concept, is an open-loop system. It requires the patient to decide how to manage the monitoring process. In reality, however, this seemingly simple task has been very difficult. ''Sensitivity'' and ''specificity'' are usually used to evaluate the effectiveness of the alarm methodology as well as the usefulness of the device.

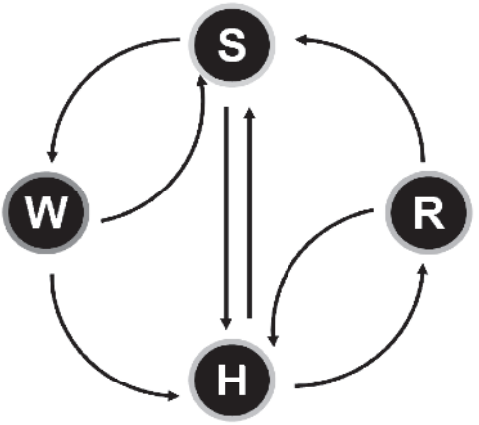
$$\text{Sensitivity} = a/(a+c) = \text{true positive}/(\text{true positive} + \text{false negative})$$
$$\text{Specificity} = d/(b+d) = \text{true negative}/(\text{false positive} + \text{true negative})$$

	Patient with hypoglycemia	Patient without hypoglycemia
Alarm triggered	True positive	False positive
Alarm untriggered	False negative	True negative

Sensitivity tells how accurately the device sounds true alarms, while specificity defines how accurately the device avoids false alarms. The concept may appear vague, but it vividly describes real situations. A sensitivity of 80% means that there are two undetected hypoglycemia events every eight times the alarm sounds. A specificity of 80% indicates that in every 10 alarms there are 2 false alarms.

The rate of decrease in sensor glucose, in combination with a hypoglycemic threshold, can be used to predict an upcoming hypoglycemic event as we have demonstrated in rats.[45] The system is based on a finite-state machine, an algorithm that permits orderly passage between a safe state, a hypoglycemic state, and two intermediate states (Figure 1.3). Ideally, this is a good approach for forecasting potential hypoglycemia, thus permitting the patient to take corrective action before it is too late. The basis for the hypoglycemia alarm is to inform the patient that if glucose continues to decrease at the measured rate, a hypoglycemia threshold will be attained in 20 min. This affords sufficient time to permit corrective action. There are also indications in rat studies that early intervention, say at 150 mg/dL, requires less glucose infusion and avoids the rapid return of the glycemia to a hyperglycemic state. However, variation in patient physiology and status of disease (e.g., sensitivity to insulin) makes this task more complicated. A more reliable model must be developed to include numerous patient-specific parameters. When one tries to implement advanced triggers of alarms, or to be rightly termed ''alerts,'' the risk of generating false alarms is significant. This is a condition that would not be tolerated by patients.

To date, there is no commercial glucose sensor system that provides complete diabetes management capability. Medtronic Minimed developed an ''open-loop'' system that utilized the data from CGMS Gold and the insulin pump. With historical insulin dosing parameters from individual patients, the new pump software can calculate a suggested insulin dosage. This suggested dosage is presented to the user as a reference.



S: *Safety state*: normal blood glucose level, without risk for hypoglycemia

W: *Time-based warning state*: blood glucose level is decreasing and expected to reach a hypoglycemia threshold (HT) in less than m_h minutes

H: *Hypoglycemia state*: blood glucose level is lower than HT

R: *Recovery state*: blood glucose level is lower than HT but increasing and expected to be higher than HT in less than m_r minutes

Figure 1.3 Finite-state machine used to control monitor response in the course of avoiding hypoglycemic events.

1.4 GLUCOSE KINETICS

There is perhaps no subject more confusing in the development of glucose sensors than the relationship between the blood glucose, measured variously as capillary glucose, plasma glucose, or whole blood glucose. The calibration of continuous monitoring systems and the subsequent assessment of their performance depends on the assumption that the two are equal. There are three factors that can contribute to this discrepancy. First, the intrinsic response time of the sensor must be considered. If the response time exceeds about 5 min, then it can be a contributing factor. Second, signal processing techniques that remove noise from the signal can cause a delay. Finally, the physiological response must be taken into account. Bergman and coworkers have studied the interactions of glucose and insulin and have developed multicompartment models to explain glucose distribution and insulin resistance.[46] For the present discussion, we use a three-compartment model shown in Figure 1.4. The sensor is placed in the middle compartment and it is assumed to be measuring accurately the interstitial glucose concentration. There are two sources of glucose found in the blood: that derived by the conversion of glycogen into glucose (G_{end}) and glucose resulting from dietary intake (G_{ex}). When glucose reaches a relatively high threshold in the blood, it can be eliminated via the kidney (see above) (G_k). Insulin can also control the uptake of glucose by cells. Long-term osmotic equilibrium is assumed to be established between the capillaries and the interstitial fluid. Of interest, therefore, is the ratio of the blood glucose (BG) to the glucose of the interstitial fluid (IG). This was first addressed definitively by Fisher and coworkers using the so-called ''wick'' technique.[11] When BG concentrations are increasing rapidly due to the ingestion of carbohydrate, the BG/IG ratio is consistently greater than unity. On the other hand, decreases in blood glucose in diabetic subjects are typically triggered by the injection

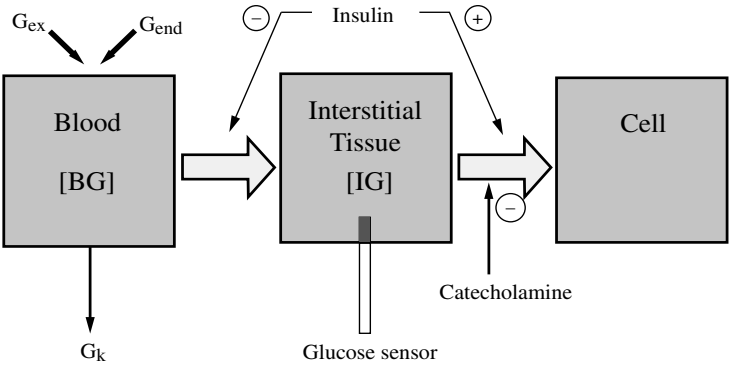


Figure 1.4 Glucose kinetics three-compartment model. G_{ex}, exogenous glucose; G_{end}, glucose produced by gluconeogenesis; G_k, glucose eliminated via the kidney; BG, blood glucose; IG, subcutaneous interstitial glucose. (See the color version of this figure in Color Plates section.)

of insulin, and the resulting response is not necessarily the second half of a ''phase shift'' or ''time lag'' as has frequently been suggested. During the decrease, the BG/IG ratio can be greater than, equal to, or less than unity. This question has been addressed in rats and in humans.[47,48] The effective ratio is determined by the placement of the sensor and by the extent of insulin resistance. The former condition (BG/IG > 1) is seen in young rats, especially when the sensor is implanted in adipose tissue, and the IG levels can remain low (in the hypoglycemic region) for extended periods of time even when the BG has recovered to normal levels. The latter (BG/IG < 1) condition is seen in old, obese, insulin-resistant rats. A ratio of BG/IG $= 1$ is achieved when the BG concentration is not changing and when insulin has not recently been administered. Thus, the ideal time for calibration is in the morning before breakfast.

The consequences of time-dependent nonunity ratios of BG/IG have also been manifested in the attempt to use fingerstick system sampling at alternate sites, that is, the arm or thigh rather than fingertips.[49] Once again, alternate sites show significant differences with respect to the capillary glucose value when glucose concentrations are changing rapidly.

1.5 EVALUATION OF SENSOR PERFORMANCE (*IN VITRO*)

Sensors should always be evaluated *in vitro*. While this is clearly not a substitute for *in vivo* testing, it is easier to diagnose fundamental problems without the complications that the biological milieu introduces. Furthermore, if they do not work reliably *in vitro*, they will not work *in vivo*. In addition to the linear dynamic range mentioned above, stability and reproducibility of characteristics in sensor production are very important. Linearity can be characterized by comparing the sensitivities (slope of the dose/response curve) at 5 and 15 mM glucose, assuming that they should not deviate by more than 10%. Stability can be measured in several different ways. Sensors can be stored dry and at room temperature between periodic sensitivity checks. This tends to

yield more optimistic results than operating the sensor constantly in a buffer at 37°C. Storing in a refrigerator is not necessarily beneficial as the sensor may suffer thermal shock because the metal (electrode) and the associated polymer layers do not have the same thermal coefficient of expansion. Extended operation at body temperature can shorten the sensor lifetime.

If reaction (1.2) is used as the basis for the sensor response, it will be necessary to establish that the response does not depend strongly on oxygen concentration and also that the sensor does not respond to endogenous interferences such as ascorbate or urate. A useful way of making this determination is to measure the percent increase (decrease) in the signal corresponding to 5 mM glucose when the physiological concentration of the interference is added. Urate as an interferent to electrochemical sensors has never presented a serious challenge. It has a stable low concentration in the body (2–8 mg/dL, ~0.15–0.5 μM in serum). Most known sensor membranes are effective in blocking its diffusion. Ascorbate, with a base concentration of 0.4–1.0 mg/dL (0.02–0.06 mM) in serum, on the other hand, can vary over a wider range because it is present in most food substances. Ascorbate is also a popular food supplement for its role as an antioxidant. For all practical purposes, testing for ascorbate interference can be done with an addition of ascorbic acid to the test buffer to a level of 0.2 mM, which gives a maximum possible interfering level. The criterion for assessment can be that the overall signal caused by ascorbate be no more than 10% of the corresponding signal for 5 mM glucose. If a mediator is used instead of oxygen, it will still be necessary to verify that no chemical reaction occurs directly between the mediator and the endogenous reducing agents. If the mediator is oxidized at a potential lower than that of peroxide, a likely occurrence, then reaction (1.4) will compete with reaction (1.4′) with the result that the measured glucose concentration will be erroneously low. There have also been reports that oxidation of endogenous species such as ascorbate can foul the electrode.[50] There have been a number of reports of electropolymerized films serving to exclude electroactive interferences.[51,52] In our experience, many of these work well for short periods (1–2 days), but then the selectivity deteriorates rapidly. In addition to the electropolymerization of phenol,[53] we have had some success with sol–gels.[54]

1.6 EVALUATION OF SENSOR PERFORMANCE (*IN VIVO*)

1.6.1 *In Vivo* Sensitivity Loss

The literature is rather vague concerning the question of what happens to the sensitivity of a sensor when it is implanted in a biological fluid. In our hands, subcutaneous sensors immediately and rapidly lose sensitivity, a process that takes place in minutes. Despite losses in sensitivity of 10–30%, these sensors will function satisfactorily over periods in excess of 4 days. Indeed, the performance of the sensor frequently improves with time. The origin of this sensitivity loss has been studied in detail and some results are shown in Figure 1.5.[55] If the sensor is removed from the tissue and quickly calibrated in buffer solution (10 min), essentially the same *in vitro* sensitivity is obtained as for the *in vivo* value. Further incubation in buffer causes the sensitivity to rise until eventually the original *in vitro* value is obtained. The important

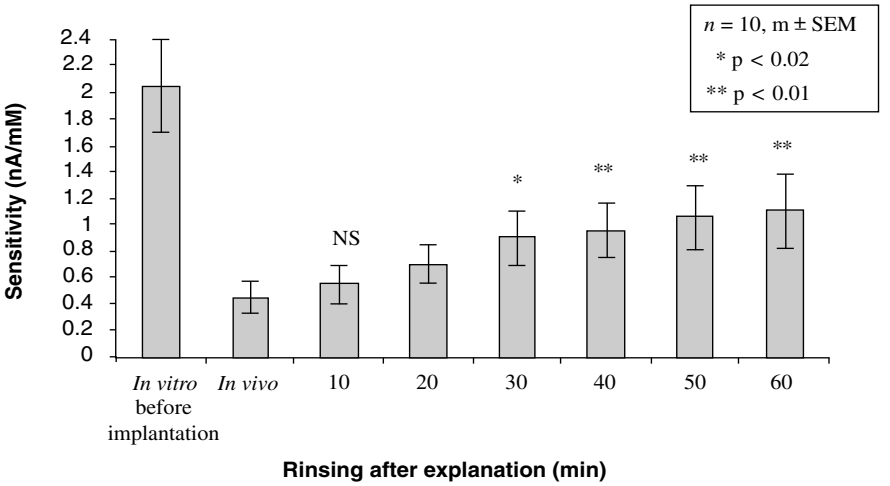


Figure 1.5 Loss of sensor sensitivity on implantation followed by regeneration on rising in glucose-containing buffer. Reprinted with permission from Ref. 55. Copyright 1996 Masson–Elsevier.

conclusion from these experiments is that the loss is specifically associated with the sensor itself and is retained even when the sensor is removed from the tissue. Since the process is overall reversible, this suggests that the cause of sensitivity loss is passive, meaning that the passage of glucose into the enzyme layer is being blocked. It is unlikely that biofilm formation or enzyme activity loss could be the cause of this very rapid sensitivity decrease and subsequent recovery on explantation.

Assuming that the regaining of the original *in vitro* sensitivity is caused by the leaching out of material from the interior of the sensor, we have examined the leachate using a proteomic approach. Rather than intact proteins, protein fragments dominate, suggesting that they are the result of proteolytic reactions at or near the sensor surface.[56] Studies in our laboratory have shown that incubating sensors with physiological concentrations of serum albumin, fibrinogen, and/or IgG, which are present in relatively high concentrations, produce very little change in the *in vitro* sensitivity.[57] On the other hand, incubation in serum can produce significant sensitivity losses.[58]

1.6.2 Calibration *In Vivo*

As noted above in the discussion of glucose kinetics, reliable calibration must eliminate the discrepancy between the tissue and blood glucose values. This suggests that calibration in the morning before breakfast will be the optimal solution. The performance of our glucose monitoring system in a diabetic patient is demonstrated in Figure 1.6,[59] where the performance of a sensor is evaluated over a period of 7 days. A key question is whether this calibration should be a one- or a two-point calibration. We have examined this question in considerable detail,[60,61] and this is also consistent with the finding of Heller and coworkers that a one-point calibration is to be preferred.[39]

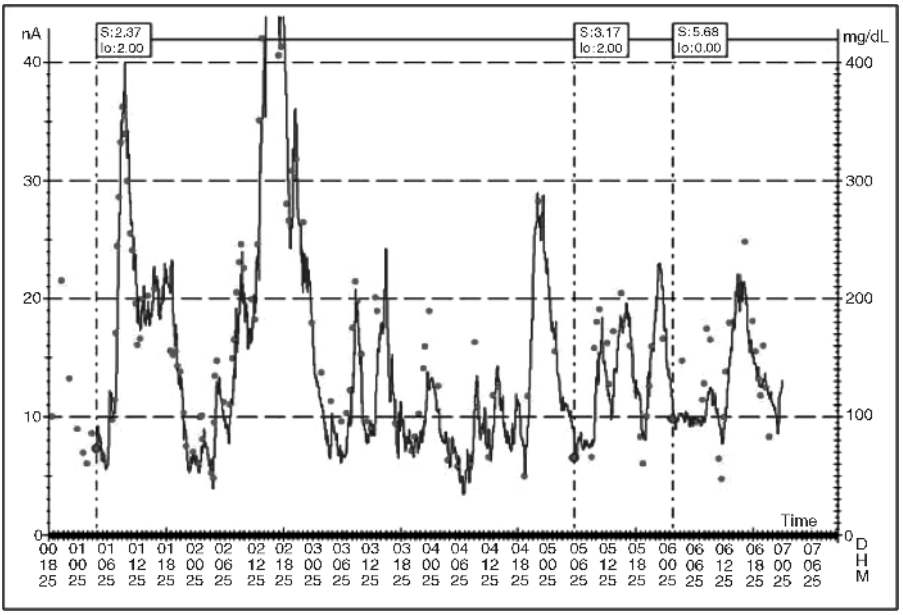


Figure 1.6 Seven-day monitoring of a diabetic patient. Points obtained from fingerstick method, solid trace — continuous sensor response. Three two-point calibrations are noted on days 1, 5, and 6.

At first glance, this conclusion seems counterintuitive. However, determining the slope of the calibration curve (the sensitivity) using two points means that the uncertainty of both points must be taken into account. In a one-point calibration, the I_0 value, that is, the current in the absence of glucose, is assumed to be a certain value with zero variance. Thus, the uncertainty of the slope of the calibration curve is determined only by one glucose measurement.

A nonstatistical method for *in vivo* sensor performance evaluation has come into use, the Clarke error grid analysis (EGA).[18] This approach was originally developed for the evaluation of fingerstick systems and is based on a conventional correlation plot of the performance of the test system with respect to a referee method (ideally a clinical analyzer). If the correlation were perfect, all points would fall on a 45° line. The area surrounding this line is divided into zones that predict the clinical consequences in terms of action taken by the patient, depending on where the measurements by the test system fall off the line. Zone A would yield a clinically accurate decision (take insulin, take glucose, or do nothing), zone B a clinically acceptable decision, and zone D a clinically erroneous decision. Figure 1.7 is an EGA of the data of Figure 1.6. It will be noted, for example, that a few points fall in zone D at a blood glucose concentration of less than about 5 mM. Such points are of concern because they represent a situation in which the patient believes that the BG value is in a safe region (greater than about 5 mM) when, in fact, it is in a hypoglycemic domain. This situation can arise if the blood glucose decreases more rapidly than the tissue glucose. Using this approach, sensors have been considered to perform adequately if the percentage of points falling in the A and B zones is at least 98–99%. Recently,

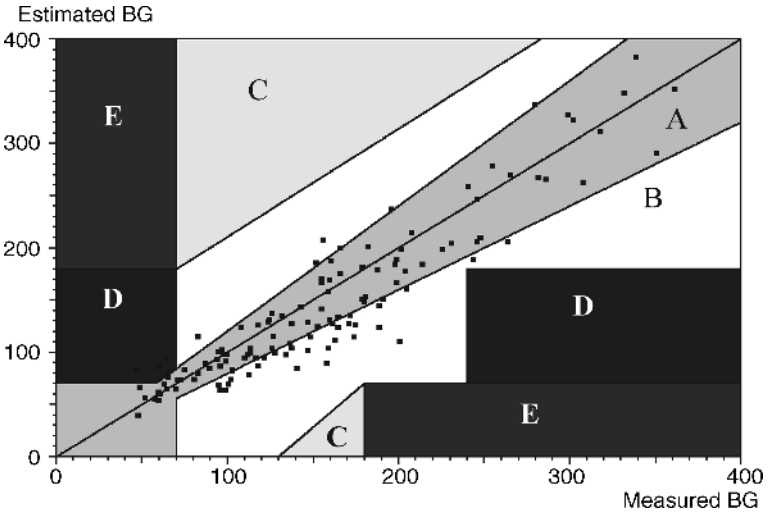


Figure 1.7 Clarke error grid analysis plot of the data of Figure 1.6.

Clarke and coworkers[25,62] have proposed an enhancement of the EGA called continuous glucose error grid analysis (CG-EGA). This approach seeks to account for the clinical consequences of the rate of change of blood glucose with respect to the rate derived from a continuous monitoring device. Two related analyses are performed: the conventional EGA, now called point error grid analysis (P-EGA) and rate error grid analysis (R-EGA). This approach was evaluated using clinical data provided by the TheraSense FreeStyle Navigator® continuous monitoring system.[25] Three regions were defined: hypoglycemia (BG $\leq$ 70 mg/dL), euglycemia (70 $<$ BG 180 mg/dL), and hyperglycemia (BG $>$ 180 mg/dL). P-EGA yields $\sim$99% of values falling in the A and B zones. However, when the rates are analyzed as a function of the three regions, accuracy drops significantly in the hypoglycemic region. For this and related devices, rate accuracy is important, particularly if the continuous monitoring system is being used to avoid hypoglycemia. It is clear that making a series of independent measurements with a fingerstick system is not the same as time-correlated measurements generated by continuous monitoring systems.

In addition, all the paired points (n) including the concentrations measured from the glucose sensor, $[\text{glucose}]_{\text{sensor}}$, and the reference glucose measurement, $[\text{glucose}]_{\text{reference}}$, in the correlation plot are used to calculate the overall mean absolute relative difference (MARD). The median MARD is the median relative difference among all the measured values.

$$\text{MARD (mean)} = \left(\frac{\left(\sum \left(\left| [\text{glucose}]_{\text{sensor}} - [\text{glucose}]_{\text{reference}} \right| \right) / [\text{glucose}]_{\text{reference}} \right)}{n} \right) \times 100\%$$

$$\text{MARD (median)} = \text{median} \left(\frac{\left| [\text{glucose}]_{\text{sensor}} - [\text{glucose}]_{\text{reference}} \right|}{[\text{glucose}]_{\text{reference}}} \right) \times 100\% \qquad (1.5)$$

Most of the sensors currently marketed or under development have MARD values in the range of 10–20%. We feel strongly that as a real-time monitoring system a MARD of no greater than 15% should be adequate (see Table 1.1).

The correlation coefficient between the two methods is always reported. For clinical acceptance, a value of 0.85 or greater may be necessary. However, because correlation coefficient can be affected significantly by a single point at extreme values, or by a lack of dynamic range, one should use caution when looking at the numbers. It has further been suggested that there should be separate performance goals for glucose sensors in the various glycemia zones.[63] International Organization for Standardization (ISO) methods for defining accuracy have also been discussed.[64]

1.7 BIOCOMPATIBILITY

The word ''biocompatibility'' has been overworked to the point of exhaustion because there are few definitive criteria to define what it means. Several chapters in this book deal in some detail with the biology associated with implants. The present operational definition of biocompatibility will focus on whether interactions with the subcutaneous tissue materially affect the proper functioning of the sensor and whether the presence of the sensor influences adversely the biological environment. Studies of sensors indwelling in the vascular bed are less numerous. The intravascular sensor is less popular mainly because it requires surgery to implant and explant, and it cannot be percutaneous for a long term because of the risk of systemic infection. It is very troublesome if a sensor malfunctions and has to be taken out. Embolization of clots is generally not a risk because clots go to the lung where they are filtered and eventually dissolve. The implant is also placed in a vein rather than an artery, because it is less painful. When a clot adheres to the sensor, however, it becomes a major problem. The sensor is walled off from the blood and is no longer accessible to blood glucose. This is, in fact, a classic biocompatibility issue. This is the representative disadvantage for an intravascular sensor, and development of sensors capable of evolving NO may help to mitigate this problem (see below). The challenge of clot formation is ever present as long as the sensor is in the vascular bed. In contrast, a subcutaneous sensor can acquire a stable state once the acute interactions with the surrounding tissue subside.

Most of the sensors implanted in the subcutaneous tissue are 200–250 μm (33–31 ga) in diameter. They are usually implanted using a guide cannula whose outside diameter is 21–23 ga (813–635 μm). This insertion process will cause some tissue injury including breaking of capillaries. Experience in our laboratories has shown, however, that the damage is very slight and an edema around the implant, typically the size of a mosquito bite, disappears after about 24 h.

There are perhaps four sources of failure or apparent failure of subcutaneously implanted glucose sensors. The first is the passive loss of sensitivity resulting from uptake of species on or into the sensor.[55] It is quite likely that these species are of relatively low molecular weight (>15 kDa) and could include protein fragments, lipids, and a variety of small endogenous molecules. This process is relatively rapid and has generally been referred to as the ''run-in'' time, lasting 2–4 h. In addition, a second necessary phase of the initial run-in time is the rehydration of sensor

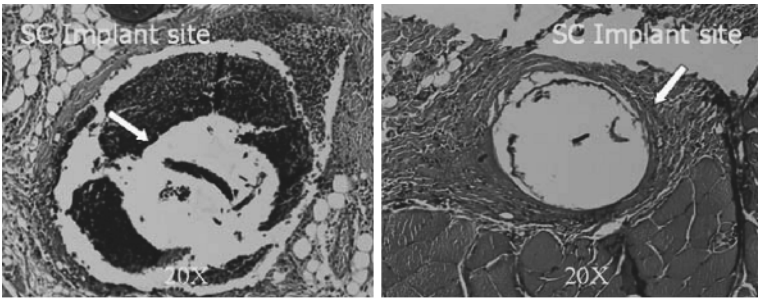


Figure 1.8 Tissue section at implant site showing the effect of NO evolution on the acute inflammatory response. (a) Sensor with no NO evolution (control); (b) no evolution for about 18 h. Reprinted with permission from Ref. 65. Copyright 2005 John Wiley & Sons. (See the color version of this figure in Color Plates section.)

membranes that have been stored dry. It takes a finite amount of time for the membranes to hydrate and for the local microenvironment to establish the required mass transfer balance before stable operation can be achieved. The second is the perturbation of oxygen and glucose levels in the tissue surrounding the sensor resulting from tissue injury and the subsequent acute inflammatory response. This latter process, although complicated, has been studied in some detail and is described elsewhere in this book. It is typically manifested in sensor instability over the first 48 h but can result in cleanup of the small amount of capillary blood that may have accumulated. Tissue sections from a rat after 3 days show evidence of macrophage formation and angiogenesis (restoration of the damaged capillaries). We have demonstrated that sensors designed to evolve NO on implantation significantly reduce the acute inflammatory response as shown in Figure 1.8.[65] Experience in a number of laboratories has shown that subcutaneously implanted sensors can function reliably for 1–2 weeks if they survive the initial several days. For long-term implants, two other issues can become important. There will be a loss of enzyme activity that will eventually affect sensor response. Finally, the capsule formation caused by the foreign body reaction may limit the access of glucose and oxygen to the sensor. This latter issue has been addressed by encouraging angiogenesis as the sensor is implanted.[66] There is, in addition, the case already discussed and related to glucose kinetics, where the sensor correctly measures the glucose, but these values do not correspond to the blood value. This question can be resolved by calibrating the sensor under the appropriate conditions. Occasionally, sudden catastrophic failure of the sensor can occur. The reasons for this are not clear, but may be due to the fracture of a capillary owing to movement of the sensor in the tissue, thus releasing some blood.

1.8 FUTURE DIRECTIONS

The development of reliable, user-friendly glucose sensing systems remains a significant challenge. Applications can be diverse, including rapid screening of subjects for diabetes (say at a mall), self-monitoring of diabetes several times a day

using test strips, continuous monitoring systems for bedside monitoring (especially appealing for monitoring children), wearable continuous monitoring systems, and monitoring of subjects (not necessarily diabetic patients) under trauma and critical care situations. The wide range of applications will demand a multiplicity of solutions very likely exploiting different technologies. It is to be hoped, for example, that truly noninvasive approaches, in which no component of the sensing system is in contact with a biological fluid, will become a reality.

ACKNOWLEDGMENTS

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