ANNUAL REVIEW OF BIOMEDICAL ENGINEERING

VOLUME 1, 1999

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MEHMET TONER, *Associate Editor*
Center for Engineering in Medicine
Massachusetts General Hospital/Harvard Medical School, Boston

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4139 El Camino Way • P.O. Box 10139 • Palo Alto, California 94303-0139

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International Standard Serial Number: 1523-9829
International Standard Book Number: 0-8243-3501-5

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Typeset by Impressions Book and Journal Services, Inc., Madison, WI
Printed and Bound in the United States of America

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Annu. Rev. Biomed. Eng. 1999. 01:153–175
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IMPLANTED ELECTROCHEMICAL GLUCOSE SENSORS FOR THE MANAGEMENT OF DIABETES

Adam Heller*
Department of Chemical Engineering and Texas Materials Institute, University of Texas at Austin, Austin, Texas 78712–1062; e-mail: heller@che.utexas.edu

Key Words biosensors, subcutaneous, amperometry, continuous monitoring

■ **Abstract** By maintaining a near normal (70–120 mg/dL) glucose concentration, diabetic patients can drastically reduce the likelihood of the occurrence of diabetes complications. In the near future, subcutaneously implanted electrochemical glucose sensors will be available to provide frequent or continuous information on which timely treatment decisions, such as insulin injection or glucose source intake, can be based, as well as timely alarm signals. The currently engineered devices are of three types: (*a*) innocuous microsensors, with actively mass-transporting areas $<10^{-3}$ cm^2, replaced twice a week by the patient; (*b*) self-contained, surgeon-implanted, transmitter-containing packages of >1 cm^2 area, operating for >100 days; and (*c*) devices transporting subcutaneous fluid to an external sensor, based on implanted microfiltration or microdialysis fibers or on iontophoretic transport of the subcutaneous fluid through the skin.

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*Consultant to TheraSense, Alameda, CA.

1523–9829/99/0820–0153$08.00

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DIABETES AND ITS PREVENTABLE COMPLICATIONS

The term diabetes describes a group of metabolic diseases manifested by inadequate control of glycemia. In people not afflicted with diabetes, the blood glucose concentration is usually in the 70- to 120-mg/dL range or ~4–8 mM. It is lower when a person is hungry and higher after a meal. In people with diabetes the range is much wider, 30–500 mg/dL or ~2–30 mM. Very low glycemia, termed hypoglycemia, may lead to fainting, coma, and even death. Persistent or frequent hyperglycemia (high blood glucose level) damages the eyes, kidneys, nerves, and blood vessels. The hormone insulin is an essential element of glycemic control.

About 16 million people in the United States have diabetes, suggesting that about 3×10^8 people worldwide may suffer from the disease. The associated annual direct and indirect cost to the US economy is about $\$10^{11}$ (66).

Diabetes results if the pancreas does not produce insulin or if the insulin released by the pancreas does not accelerate the uptake of glucose by cells. Type I diabetes is an autoimmune disease in which the body's immune system destroys the insulin-producing cells of the pancreas. It affects ~10–15 million people worldwide and is most frequently diagnosed in children and in young adults. The lives of type I diabetic patients can be sustained only with injections of insulin, which, after meals, lowers the glucose concentration in the blood. In type II diabetes, which usually afflicts mature, often overweight people, the insulin produced does not adequately accelerate the uptake of glucose by the cells of the human body. About 60% of the people with type II diabetes have hypertension (66).

Diabetes has acute and chronic effects. The acute effects, including fainting, coma, and death, result from hypoglycemia. Acute effects are dramatic but rare. The chronic effects result from the persistent maintenance of higher-than-normal glycemia, practiced by a large fraction of the patients to avoid the acute effects of hypoglycemia. Persistent maintenance of higher-than-normal blood glucose concentrations damages the retina, kidneys, nerves, and circulatory system. It is the dominant cause of the reduced longevity of diabetic people. It is the leading cause of blindness among US adults 20–74 years of age, with 12,000–24,000 new cases recorded each year, and also of end-stage renal disease, with ~20,000 cases recorded annually. It accounts for 35% of dialyses and kidney transplants and is the cause of the majority of limb amputations (66).

It has long been assumed, but not proven, that if diabetic patients maintain normal or near-normal glycemia, the likelihood of the occurrence of complications of diabetes decreases. Dramatic evidence for the validity of this assumption was obtained in the Diabetes Complications and Control Trial of the US National

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Institutes of Health (25). The report on this trial, released in 1993, showed that controlling glycemia within tight, more nearly normal limits, can drastically reduce the incidence of eye disease (retinopathy), kidney disease (nephropathy), nerve disease (neuropathy), and vascular disease, cutting their occurrence by 60% or more (25, 28, 29). Although most of this study focused on people with type I diabetes, the conclusions were similar for people with type II diabetes (26, 27, 68).

The absence of elevation of glycosylated hemoglobin, hemoglobin A1c, which accumulates at high glucose concentrations, is an indicator of stable and well-controlled blood glucose concentration. Chronic complications, loss of sight and limbs, and shortened life expectancy are prevalent among patients with persistent or frequent hyperglycemia, resulting in a high level of hemoglobin A1c (66).

Although the results of the Diabetes Complications and Control Trial were well publicized and practically all the diabetic people in the United States were made familiar with its implications, the fraction of patients who switched to tight control was small. The added cost for intensive monitoring of ~$2/day, for 3–5 assays/day vs 1–2 assays/day, was not affordable to all patients. In addition, the pain associated with the finger-stick assays deterred patients from frequent monitoring.

Although the advantage of frequent monitoring was established <10 years ago, research on continuously functioning implanted monitoring devices had already started in the 1960s. The first such system, called the "Biostator," was engineered by Miles Laboratories. The hospital bedside unit routed a by-stream of blood to an external monitoring unit. Today, Via Medical manufactures a system that samples a by-stream of blood at 5- to 10-min intervals.

The early objective was the "artificial pancreas," a feedback loop consisting of the implanted glucose sensor, an insulin pump, and an actuator releasing insulin at high glycemia (1, 19, 89, 90). Although the objective remains valid and progress toward it is being made (40, 91, 92), a less complex, continuous, or frequent glucose monitor not connected to an insulin pump would better the lives of nearly all type I diabetic patients and of a large fraction of the type II diabetic patients (66). Assisted by such a device, they could live a full-term life, avoiding both the debilitating chronic complications of the disease and its acute risks. The monitor would tell the patient on demand his or her glycemia and its direction of change and would provide an alarm signal upon actual or impending deviation from the desired glycemic range. The device would have to be provided at an acceptable cost, be painless, and be unobtrusive.

PRINCIPLES OF CONTINUOUS MONITORING OF THE CONCENTRATION OF GLUCOSE IN VITRO

Glucose concentrations can be monitored intermittently or continuously in vitro by electrochemical and photonic means. The electrochemical methods are based on amperometry or potentiometry, whereas the main photonic methods (47) are

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polarimetry, near-infrared absorption spectroscopy (5, 33, 35, 65), near-infrared Raman spectroscopy (12), fluorometry, and refractometry. Whether electrochemical or photonic, the monitoring method can be of three types: nonreactive, meaning that glucose is not reacted in the monitoring process; reversibly reactive, meaning that glucose is reversibly binding with a reactant, usually displacing, upon binding, a competing reactant; or irreversibly reactive, meaning that glucose is being consumed in the sensor reaction. In the latter case, glucose is irreversibly converted to a reaction product such as gluconolactone, when the irreversible reaction is glucose oxidation. In the case of an irreversible reaction, flux of glucose to the reaction zone is required. The flux, and therefore the reaction rate, increases linearly with the glucose concentration.

In the nonreactive measurements an intrinsic property of the glucose solution, such as its refractive index or its angle of rotation of polarized light, is measured. In the reactive equilibrium measurements the equilibrium of a reversible glucose-involving thermodynamic equilibrium is shifted when the glucose concentration is changed. The shift results in a measured change of color, pH, or conductivity. In the reactive, transport-requiring measurements, glucose is converted irreversibly to a product at a catalytic site, acting as a glucose sink. This review focuses on monitors based on reactive electrochemical measurement devices, which are likely to be the first available. The reactive electrochemical methods, in which the concentration of glucose has been continuously monitored in vitro, include amperometry, potentiometry, chronoamperometry, and chronopotentiometry, as well as measurement of conductance and impedance. However, thus far, only amperometric sensors have functioned effectively in vivo.

TRANSPORT-DEPENDENT REACTIVE GLUCOSE ASSAYS

In the irreversible, transport-dependent reactive glucose assays, the glucose source is the monitored biological fluid. An electrode surface or a zone in its proximity where the glucose is reacted constitutes a glucose sink. Most often the sink reaction is the oxidation of glucose. Glucose is oxidized at a measured rate k, which is proportional to the signal s. In the amperometric assay the flux of glucose to the sink electrode depends only on its concentration; when the concentration is constant, the current produced in the transducing electrode is time invariant. This is, however, not the case if the sensor is progressively fouled by a glucose-flux–blocking deposit or if glucose-consuming cells or organisms settle or grow on or in the proximity of the sensor. To avoid fouling and adhesion of glucose-consuming cells, the solution-contacting surface of the sink compartment must be coated with a bioinert material or be made of a material that is predictably fouled.

In the absence of fouling or glucose consumption by organisms or cells, the flux to the sink increases linearly with the concentration of glucose. When all of the influx of glucose is reacted, then the measured conversion rate k is glucose-

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flux and thus glucose-concentration controlled. However, when the rate is limited by the kinetics of the sink reaction, k is not glucose-flux controlled and is not proportional to the glucose flux or concentration. In this case only a fraction of the influx of glucose is reacted, and the sensor "saturates"; the signal no longer increases, or increases only slightly, with the glucose concentration. To extend the sensed glucose concentration range to all relevant concentrations, including the highest encountered at severe hyperglycemia (30 mM), it is usually necessary to limit the flux of glucose to the sink. This is done by inserting a flux-limiting membrane between the monitored fluid and the reactive sink compartment. The flux of glucose through the membrane is defined by the permeability of the membrane to glucose, which is usually constant, and by the concentration of glucose in the monitored solution c. If all of the transported glucose is consumed in the sink reaction, then the flux of glucose through the membrane to the sink varies linearly with c, and the rate of the sink reaction is proportional to c.

It is usually possible to reduce the permeability of the glucose-transport–limiting membrane sufficiently to provide for a linear dependence of k on c. However, reducing the permeability carries two penalties: first, the specific sensitivity, which is the change in the sensor's signal, ds, per unit area dA, ds/dA, which is proportional to dk/dA, is reduced; second, the response time of the sensor, τ, is increased. The consequence of the first penalty is that to maintain sufficient sensitivity, the area of the sink must be increased; the consequence of the second penalty is a delay between the measured and actual glucose concentration. The error in the glucose readings, ε, introduced by τ, is $\varepsilon = \tau\, dc/d\tau$: the error is proportional to the delay and to the change in glucose concentration per unit time. Because τ only approaches, but rigorously does not attain, its exact actual value, it is customary to specify a time $\tau_{90\%}$, which is the time required for the signal to approach within 10% of its ultimate value at a given concentration c.

The utility of an implanted sensor thus depends on (*a*) the upper limit of its sensing range (which determines whether or not hyperglycemia can be monitored); (*b*) its specific sensitivity (sensitivity per unit glucose transporting area), which defines the extent to which the sensor can be miniaturized; and (*c*) its response time. By increasing the rate of the sink reaction per unit sink area, it is possible to reduce simultaneously the area, to shorten the response time, and to monitor the glucose concentration through the full-range relevant glycemic range. Furthermore, because the blood glucose concentration of brittle diabetic patients can change by 2 mg/(dL/min) and because an error of 20 mg/dL can lead to potentially harmful treatment, such as injection of insulin when the glucose level is already low, the delay $\tau_{90\%}$ must not exceed 10 min.

AMPEROMETRIC GLUCOSE SENSORS

With few exceptions, all electrochemical in vivo sensors are at this time enzyme based and nearly all use glucose oxidase. The nonenzymatic electrooxidation of glucose at neutral pH is impractical because of fouling of the electrocatalyst,

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usually a platinum group metal. The enzyme can be stabilized by immobilization on surfaces and is particularly stabilized by immobilization in hydrated, microporous silica xerogels (17, 37).

The most widely studied implanted amperometric sensor originated in the work of Updike & Hicks (95), who measured the oxygen consumption by glucose-reduced $FADH_2$ centers of glucose oxidase, using the oxygen electrode of Clark & Lyons (20). There are several O_2/H_2O_2 couple-based sensors with similar chemistries but greatly differing in their life, size, and ease of implantation. Their common reaction is the glucose oxidase-catalyzed oxidation of glucose by dissolved O_2, whereby gluconolactone hydrolyzed to gluconate and H_2O_2 are produced. The larger of these sensors (>1 cm^2 solution-contacting area) contains a massive excess of immobilized glucose oxidase (over that required to convert, at the time of implantation, the entire glucose flux). This excess provides longevity, the operational life of the sensors exceeding 100 days and, potentially, reaching years (4, 31, 96, 97). Longevity is also provided by electrocatalyst-activated carbon particles, a slurry of which is confined in a subcutaneously implanted sack. The slurry could be replaced, using a hypodermic needle, without replacing the sack (9, 10, 99, 100).

A second group of O_2/H_2O_2 couple-based amperometric-glucose sensors is designed for subcutaneous implantation and replacement by the patient once or twice per week. At the theoretical design limit, their size is controlled by the area of the blood-contacting membrane that is required for the permeation of enough oxygen for reacting the entire influx of glucose. Because the concentration of O_2 in the subcutaneous fluid is only $\sim 10^{-4}$ M, ~300 times lower than the glucose concentration reached in hyperglycemic patients (3×10^{-2} M), the area required for O_2 permeation, 10^{-1}–10^{-2} cm^2, is ~100-fold larger than that required for glucose permeation. If oxygen is not consumed in the sensing reaction and its permeation is not required, then the area of the membrane that now permeates only glucose can be much smaller, (23, 24, 55, 75, 83–85, 98).

When protected by NAFION™, O_2/H_2O_2 glucose electrodes and their associated Ag/AgCl counter/reference electrodes are remarkably stable, withstanding dry heat of 120°C (61–64). NAFION™ is, however, fouled through mineralization by calcium, which reduces the glucose and oxygen flux. The process of its biomineralization is alleviated, but not prevented, by pretreatment with Fe^{3+} (57). Electrodes of 0.4 mm diameter with fluid-contacting areas of 1.2 (10^{-2} cm^2) (13) have been tested in human volunteers (72). In these electrodes the enzyme was immobilized on a platinum wire, an electrocatalyst for oxidation of H_2O_2. O_2/H_2O_2 couple-based subcutaneous glucose sensors have also been made with good reproducibility on silicon (14, 48–50) and on flexible polymers with metal electrodes, runners, and contacts (42). Glucose oxidase was also immobilized on the inner surface of a needle (102, 103).

In a third group of glucose concentration-monitoring systems, the subcutaneous interstitial fluid is transported to an external sensor. The fluid is drawn by suction through an implanted microfiltration fiber (6–8). Alternatively, it diffuses

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into a solution that is forced to flow through a microdialysis fiber. From the fiber the solution flows to the sensor (15, 22, 34, 38, 43, 44, 46, 54, 56, 58, 59, 70, 80, 86, 101). In microdialysis, equilibration with the subcutaneous fluid is only partial, and the glucose concentration difference between the flowing solution and the subcutaneous fluid critically depends on the flow rate and on the extent of fouling of the fiber by proteins and adhering cells. However, cell growth on the fibers perturbs the measurements. In the fiber-based systems, the problems of growth of glucose-consuming organisms in the fibers and the requirement of either microsurgery or use of large 16- to 18-gauge needles for fiber insertion remain issues to be solved.

In a fourth group of O_2/H_2O_2 couple-based systems, the subcutaneous fluid is transported across the skin through iontophoresis (52, 78, 93). For the iontophoretic transport to be fast enough for the time delay $\tau_{90\%}$ to be <10 min, the current density must be increased, however. A high enough current density may damage the skin, causing inflammation. To avoid excessive damage, the iontophoretic skin patch is moved daily from site to site.

DIFFUSIONAL MEDIATOR-BASED SENSORS

The disadvantages resulting from the consumption of oxygen in implanted sensors are avoided by replacing the O_2/H_2O_2 couple with a fast redox couple. Ex vivo single-use electrodes based on diffusing redox couples are now in wide use in finger-stick assays (16,51). The diffusing redox couple loads electrons in the redox centers of the glucose-reduced enzyme and unloads them at the electrode, obviating the need for oxygen. In vivo sensors were built with diffusional mediators such as the ferrocene/ferricinium carboxaldehyde or the dimethyl ferrocene/ferricinium redox couple (18, 67, 71, 91). Here an important engineering issue is the containment of the potentially leachable mediator. One of these electrodes uses the $Fe(CN)_6^{3-/4-}$ couple behind a polyanionic NAFION™ membrane, which slows leaching (39).

"WIRING" OF REDOX CENTERS OF ENZYMES TO METAL ELECTRODES

Networks of cross-linked electron-conducting macromolecular "wires" can be used to connect electrically the reaction centers of enzymes to electrodes (36, 77). The networks to which the redox enzymes are covalently bound swell to hydrogels that are both electron conducting and permeable to water-soluble biochemicals. When glucose oxidase is covalently bound to the cross-linked electron-conducting polymer, its FAD/$FADH_2$ reaction centers are electrically "wired," and glucose is directly electrooxidized on the gel-coated electrodes.

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Oxygen is not consumed, and diffusional mediators are not involved in the reaction. Electrooxidation of glucose now involves (*a*) transfer of electrons (and protons) from glucose to FAD reaction centers of glucose oxidase, which are reduced to $FADH_2$; (*b*) transfer of electrons from the $FADH_2$ centers to the "wiring" electron-conducting hydrogel; and (*c*) transport of electrons through the hydrogel to the electrode. The second and third steps are enabled by the motion of tethered segments of the cross-linked redox polymer network (2, 3, 76). Motion of segments of the giant network allows the redox centers, which are tethered to the cross-linked polymer network, to approach each other sufficiently ("collide") for transfer of electrons. Through such collisions, electrons flow from the protein-buried $FADH_2$ centers to the redox centers of the polymer network. The collisions also lead to exchange of electrons between reduced and oxidized centers of the network, which is the underlying cause of the electron conduction by the water-swollen networks. Thus, hydration of the cross-linked redox polymer has two effects, both essential in glucose sensing. It enhances the movement of segments, which are, in the absence of hydration, tightly held by electrostatic (ionic and dipolar) interactions, and it makes the cross-linked polymer permeable to water-soluble glucose and gluconolactone, the electrooxidation product (76). Hydration, and with it the electron diffusion coefficient, increase with the charge density on the polymer in the wired enzyme systems.

STRUCTURES AND ENGINEERING ISSUES OF IMPLANTED AMPEROMETRIC SENSORS

Surgically Implanted Long-Lived Sensors

The long-lived sensors (31, 32, 95, 96, 97) are relatively large. At least one of their dimensions is 1–5 cm, and in some sensors all three dimensions are >1 cm. The implanted packages comprise, in addition to the electrodes of the sensor (working, counter, and reference), a potentiostat, a battery, and a transmitter. Their advantage is the absence of a skin-traversing wire and the feasibility of sealing all components in a single package. They are, however, implanted and replaced by a surgeon, not by the patient. Maintenance of their fluid-contacting surface free of cells is difficult. Because cells, which grow on most implants, consume glucose, the concentration seen by the sensor may differ from the actual concentration. Implantation of the sensor leads to inflammatory reaction, with recruitment of circulating granulocytes and invasion of tissue macrophages. This stage is followed by building fibrocytic tissue, supported by an enhanced blood supply. Finally, a mature stable capsule develops (characterized by relatively avascular fibrous tissue) and attempts to isolate the foreign object from the body (97). Unless the fraction of the glucose flux passing the layer of resident cells is known and is constant or is modeled mathematically so as to allow its prediction, the glucose concentration below the cell layer, measured by the serum, is less than the concentration in the subcutaneous fluid.

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Patient-Replaced Microsensors

The 3-day glucose electrodes are innocuous microdevices, implanted and replaced by the patient (13, 22, 24, 72, 98). Their diameter is 300–400 μm, small enough that the patients do not feel their presence. Their surfaces are designed to lessen or eliminate the adhesion of cells (69, 73, 74).

Monitors with External Sensors

When the subcutaneous fluid is routed to an external amperometric sensor through an implanted microfiltration fiber or an implanted microdialysis fiber, the burden of fouling and cell growth is shifted from the sensor to the fiber. In systems based on the use of a microfiltration fiber the implanted end of the fiber is sealed, and the fiber is connected to the externally worn sensor compartment, which is connected to an evacuated cylinder, into which the fluid is sucked (6–8). The capillary flow is not excessively affected by the change in the pressure difference when the evacuated container is replaced daily. In microdialysis, one terminus of the implanted fiber is immersed in a physiological buffer solution and the other is in the sensor compartment (15, 22, 34, 38, 43, 44, 56, 58, 59, 70, 80, 86, 101). The sensor compartment is then connected to an evacuated container, as it is in the microfiltration system. Unlike the microfiltration system, where the fluid flowing in the fiber is the subcutaneous fluid, in microdialysis the subcutaneous fluid is diluted with the buffer. The process depends on the time-dependent permeation of glucose across the fiber, whereby the concentration of glucose in the fiber may approach, but not reach, the concentration in the subcutaneous fluid. Knowledge and constancy of the dilution depend on the permeability of the fiber to glucose, on the area of the fiber contacting the subcutaneous fluid, and on the flow of the fluid in the fiber. The resistance to fluid flow into the fiber is lower for the microfiltration fibers, which have larger pores, than it is for the microdialysis fibers.

One of the key objectives in all systems with external amperometric sensing is acceleration of the transport of the subcutaneous fluid to the exterior measurement chamber, that is, reduction of the sensing-related delay. A key determinant of this delay is the "dead volume," which is the aggregate volume of the fluid that is not in equilibrium with the subcutaneous fluid at the instant of measurement. The greater the dead volume, the more fluid must be transported for the reading to be valid. At fixed flow rate, the delay $\tau_{90\%}$ increases with the dead volume.

GLUCOSE TRANSPORT-LIMITING MEMBRANE

The glucose transport-limiting membrane is a key element in all implanted glucose-sensing devices (23, 24, 32, 45, 88). It defines the apparent Michaelis constant of the device, k_M^{app}. In turn, the constant defines the upper limit of the glucose concentration range over which an implanted sensor responds. When

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oxygen is not consumed in the sensing reaction and in the absence of a glucose-transport-limiting membrane, k_M^{app} is typically 5 ± 3 mM. When oxygen is consumed in the sensing reaction and in absence of a glucose-flux-limiting membrane, k_M^{app} is smaller by 2 orders of magnitude, being limited by the flux of oxygen to the reaction zone (45).

The requirements of the glucose transport-limiting membrane depend on the intended sensing range. If a sensor is designed to detect only hypoglycemia, then the requirements of the membrane can be relaxed. Monitoring of the full range of glucose concentrations encountered in diabetes imposes, however, strict demands on the flux-limiting membrane. These demands become severe when oxygen is consumed in the sensing reaction. Maintenance of high sensitivity and short response time necessitates that a membrane with a relatively large O_2-permeating surface be used, the concentration of dissolved oxygen in serum being 20- to 100-fold lower than the concentration of glucose at normal glycemia and up to 300-fold lower than the concentration of glucose at severe hyperglycemia. The condition for amperometric transduction through the glucose oxidase catalyzed reaction, in which one atom of oxygen is consumed per arriving glucose molecule, requires that the flux of oxygen molecules to exceed half of the glucose flux, even if the hydrogen peroxide produced is decomposed to water and O_2. For this reason, a prime objective of the hydrogen peroxide or oxygen partial pressure-sensing implanted glucose sensors is the development of oxygen permeable membranes, which are designed to restrict severely the influx of glucose but not of oxygen. Alternatively, a large area oxygen-permeating membrane is combined with a small area glucose-permeating membrane to provide a short response time. A combination of two membranes allows the reconciliation of the demand of a short $\tau_{90\%}$ with the balancing of the influx of oxygen with the influx of glucose (32).

In implanted sensors that do not consume oxygen, based on the wiring of glucose oxidase, a single membrane of 5×10^{-4} cm^2 area provides a response time of 90 s even when the membrane of the sensor restricts the flux of glucose sufficiently to monitor the full, 2- to 30-mM, glycemic range encountered in diabetes (24, 98).

BIOCOMPATIBLE INTERFACE

The biocompatible layer contacts the tissue in which the sensor or fiber is inserted. The severity of the requirements of the biocompatible layer is a function of the intended life of the implanted device. In the strongly fouling environment of the blood stream, sensors can be operated in a stable mode for a few hours with cellulose acetate or polyether-polyethersulfone copolymer membranes (53). The most severe requirements are imposed when the intended life of the sensor is >30 days. Partial overgrowth by cells is seen after 1 week, and after 30 days the implant is usually completely overgrown by cells (96). Overgrowth leads to

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uncertainty in the glucose flux to the sensor caused by the consumption of glucose by the cells populating the sensor surface. The uncertainty is aggravated by the oxygen consumption of the cells when oxygen is consumed in the sensing reaction. Short-term fouling, over periods of hours, depends on local flow conditions and can be significantly reduced by siting the sensor in an appropriate flow environment, the moving fluid film affording protection against fouling (81).

The requirements of the biocompatible interface are relaxed when the intended period of use is only 3 days. In this case, the likelihood that the surface will be overgrown with a glucose-consuming layer of cells is reduced. To avoid overgrowth in the 3-day period and also to avoid the adhesion of protein or platelets or the attachment of inflammatory response-related cells, the implant is coated with a nonfouled hydrogel, such as a hydrogel formed by cross-linking polyethylene oxide (22, 73, 74, 98). Polyethylene oxide–based hydrogels can be formed in situ at room temperature and under physiological conditions by photocross-linking acrylated polyethylene oxide or by chemical cross-linking of acrylate or other reactive function-terminated polyethylene oxide chains (69). The effectiveness of polyethylene oxide–based gels is seen, for example, in a study of Quinn et al (73). In this study, Pellethane™ samples, with and without a polyethylene oxide hydrogel overcoating, were implanted subcutaneously in male Sprague-Dawley rats for 7 days. Extensive growth of cells was observed on the samples without polyethylene oxide coatings, whereas the Pellethane samples were encapsulated with tissue consisting of macrophages, neutrophils, foreign body giant cells, fibroblasts, and collagen. However, practically no cells were found on samples with polyethylene oxide coatings. The cross-linked polyethylene oxide hydrogels are examples of glucose permeable materials to which cells do not adhere and on the surface of which cells do not grow. Other examples of such materials are polymers comprising natural polar functions of phospholipids (88).

The longevity of the microfiltration- or microdialysis-based monitors is also defined primarily by the fouling and bacterial contamination of the fibers and system compartments and secondarily by infection and inflammation at the site of the implant. Growth of any organism in the microfiltration or microdialysis system, or of any cell on the microfiltration or microdialysis fiber, lowers the concentration of glucose and makes relating the subcutaneous glucose concentration to the signal difficult, if not impossible.

PRECALIBRATION, ONE-POINT CALIBRATION IN VIVO, AND TWO-POINT CALIBRATION IN VIVO

When the function relating the signal s and the concentration c is both known and unchanged after implantation or it changes predictably after implantation, then a precalibrated sensor does not require recalibration after its implantation. When the function relating s and c changes upon or after implantation, then the

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sensor must be recalibrated in vivo. Calibration requires measurement of s for at least two values of c, and, usually, blood samples must be obtained and analyzed at two levels of glycemia. Most of the reported amperometric O_2/H_2O_2 couple-based in vivo sensors have substantial "background" currents, associated with unwanted, often unidentified electrode reactions of compounds other than glucose. The background current prevents their one-point in vivo calibration (see below). In the absence of a one-point calibration, the patient cannot promptly confirm that the implanted sensor operates properly. When one-point calibration is not possible, confirmation and, when necessary, recalibration require that two blood samples be withdrawn for assay: one when the patient's glycemia is higher and the other when it is lower. If, however, s depends exclusively on c and is not affected by the presence or absence of any other chemical of variable concentration in the subcutaneous fluid, then either the origin or a fixed and known intercept is a valid second calibration point. In this case the implanted sensor can be calibrated by one-point calibration, requiring the assay of only one sample of blood. Miniature 3-day sensors calibrated by such one-point calibration have been reported (23, 24, 83, 85, 98).

Passage of the calibration curve through the origin, which enables the one-point calibration, requires that electrooxidation of the common electrooxidizable species, the concentration of which can vary in serum, particularly of urate, ascorbate, and acetaminophen, not contribute substantially to the current. The electrooxidation current of most interferants can be reduced by lowering the redox potential of the glucose oxidase wiring redox polymer in the wired enzyme-based sensor. It can also be reduced by reducing the permeability of the glucose-transport-limiting membrane to these interferants.

CLINICAL IMPLICATIONS OF MONITORING GLYCEMIA IN THE SUBCUTANEOUS FLUID

If the blood glucose concentration neither rises nor declines, then the glucose concentrations in the blood and in the subcutaneous fluid are similar, or, if not exactly similar, they are related through a proportionality constant (11, 30, 41, 79, 82, 87, 94, 98). During periods in which the blood glucose concentration rises or drops, the two concentrations differ.

The relationship of the difference between a sensor-measured glucose concentration and the true glucose concentration is often expressed by a grid proposed by Clarke et al (21). The zones in this grid are lettered A to E. In zone A the measurement correctly reflects the actual glucose concentration, whereas in zone E the error of the measurement is such that if a clinical decision were based on it, the patient might be harmed. A difference of 20% between the actual and the measured glucose concentrations is considered as unlikely to lead to an incorrect clinical decision. Greater differences must, however, be avoided, particularly at

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or near hypoglycemia. The source of the difference between the actual and the measured glucose concentrations can be sensor related or physiological. A true physiological difference exists when the glucose concentration rises or falls, the difference being a function of the rate at which the glucose concentration changes; the difference is approximately proportional to the slope, dc/dt.

When the blood glucose concentration is forced to decline at 6 mg/(dL min), the magnitude of the transient blood–subcutaneous fluid difference can approach the measured concentration. For example, Schmidtke et al (83) found that when insulin (0.5 U/kg) was intravenously injected in the rat, the subcutaneous concentration was transiently higher, by as much as 84%, than the blood glucose concentration, the difference reaching its maximum 25 ± 7 min after the injection. Even though the difference was large, they were able to model it mathematically. The modeling provided an algorithm for translating the measured subcutaneous glucose concentration to the blood glucose concentration at the instant of the subcutaneous measurement. Because the translation required knowledge of dc/dt, its quality depended on the frequency of sampling and thus on the $\tau_{90\%}$ of the sensor. For sensors with $\tau_{90\%}$ of 2 min the average difference between the blood concentrations and the subcutaneous concentrations during the 40-min period after the injection of insulin was reduced from 84% to 29%. Figure 1 shows the glucose concentrations, measured in the rat by a pair of intravenously and subcutaneously implanted microsensors, and the model-predicted blood glucose concentration, computed from the subcutaneous glucose concentrations and its rate of change.

Under normal conditions, after intramuscular insulin injection in a type I brittle diabetic chimpanzee, the fastest decline in a series of five experiments was 1.8 mg/(dL min) (98). Although the resulting difference between the blood and the subcutaneous glucose concentrations was readily measurable, the magnitude of the difference was unlikely to lead to an improper clinical decision by the standards of Clarke et al (21). In these experiments the subcutaneously implanted miniature wired glucose oxidase electrode was operated in conjunction with an on-the-skin ECG Ag/AgCl electrode. The chimpanzee was unconstrained and was trained to wear a small electronic monitor on her wrist and to present her heel for obtaining capillary blood samples. In five sets of measurements with five sensors, averaging 5 h each, 82 capillary blood samples were assayed, their concentrations ranging from 35 to 400 mg/dL. After one-point calibrations were performed at $t = 1$ h, the rms error in the correlation between the sensor-measured glucose concentration and that in capillary blood was 17.2%, 4.9% above the intrinsic 12.3% rms error of the Accu-Chek II® reference, through which the illness of the chimpanzee was routinely managed (98). The capillary blood and the subcutaneous glucose concentrations were statistically indistinguishable when the rate of change was <1 mg/(dL min). However, when the rate of decline exceeded 1.8 mg/(dL min) after insulin injection, the subcutaneous glucose concentration was transiently higher. Two of the five runs are shown in Figure 2, and the full correlation is shown in Figure 3.

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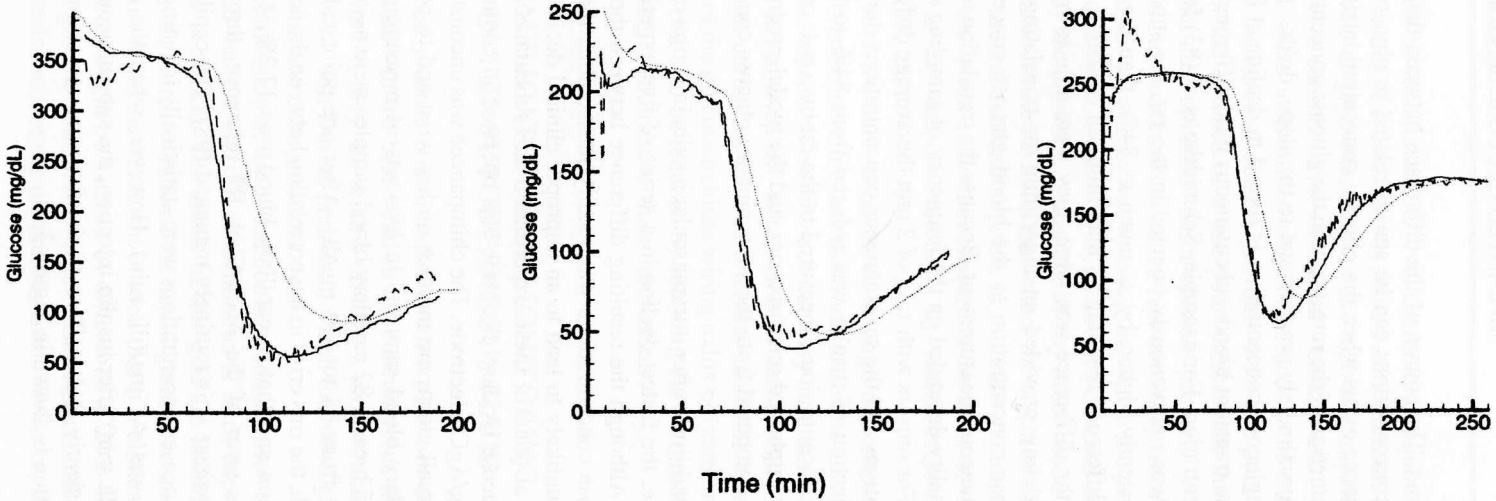

FIGURE 1 Effectiveness of the model translating subcutaneous to venous blood concentration. The rat has two miniature sensors, one in its jugular vein (*solid line*) and one subcutaneous sensor (*dotted line*). Following insulin administration the venous and subcutaneous concentrations differ transiently by as much as 80%. The mathematical model–based algorithm translates, nevertheless, correctly the subcutaneous to venous blood glucose concentration *(broken-dashed line)*.

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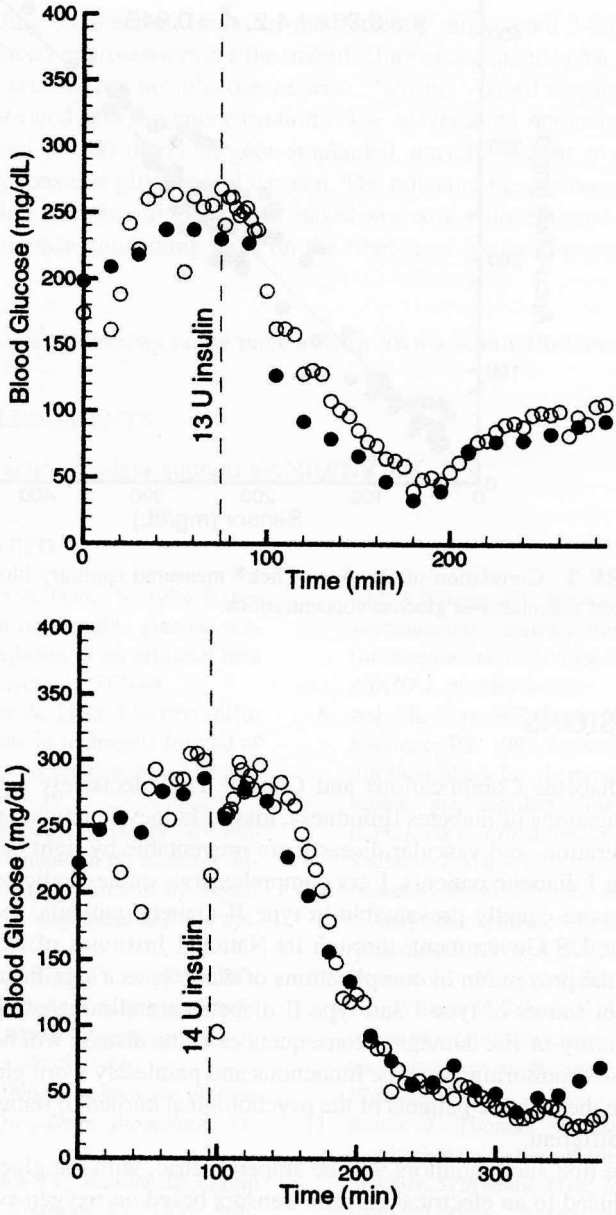

FIGURE 2 Accu-Chek® (*filled*) and sensor (*open*) readings for two experiments with one-point calibration at t = 1 h.

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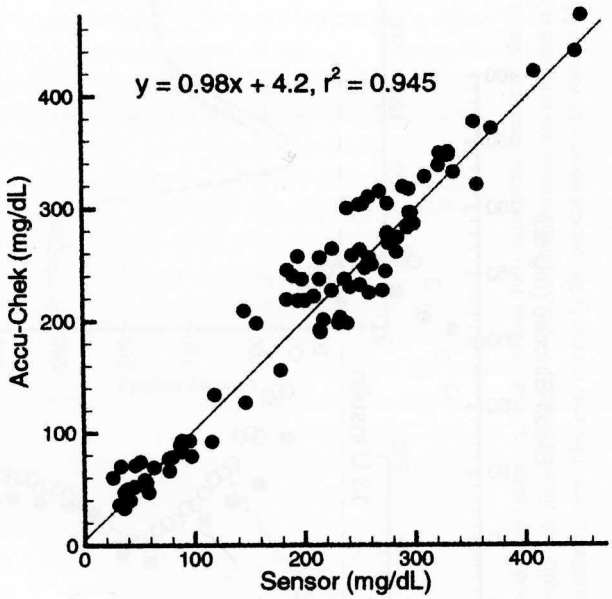

FIGURE 3 Correlation of the Accu-Chek® measured capillary blood and the sensor-measured subcutaneous glucose concentrations.

CONCLUSIONS

The Diabetes Complications and Control Trial decisively established that the complications of diabetes (blindness, loss of kidney function, amputations, nerve degeneration, and vascular disease) are preventable by tight control of glycemia in type I diabetic patients. Less comprehensive studies indicate that the complications are equally preventable in type II diabetic patients. Industry, academia, and the US Government, through its National Institutes of Health, have recognized the prevention of complications of diabetes as a significant objective. Until the root causes of type I and type II diabetes are eliminated, the life-shortening and quality-of-life damaging consequences of the disease will be avoided through glucose-monitoring systems. Innocuous and painlessly worn glucose sensors will relieve the diabetic patients of the psychological burden of remembering that they are "different."

The first such monitors will be amperometric, with the glucose concentration transduced to an electrical current. Sensors based on oxygen-consuming glucose reactions will initially alert patients to low glucose concentrations. The non–oxygen-consuming sensors to follow, such as those based on electrically connecting ("wiring") reaction centers of glucose oxidase to an electrode, will provide the patients with real-time information on their actual glycemia, whether

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normal, high, or low, and will alert them not only to hypoglycemia but also to hyperglycemia.

Outstanding engineering issues of the currently engineered 3-day innocuous patient-replaced microsensors are the manufacture at acceptable cost, and, for O_2-consuming sensors but not glucose oxidase "wiring"-based sensors, the monitoring of elevated glucose concentrations. The outstanding engineering issue of the long-lived (>100 days) surgeon-implanted sensors is their overgrowth by cells, which consume glucose and oxygen. The outstanding engineering issues of microfiltration or microdialysis fiber-based systems with external sensors are growth of glucose-consuming cells on the fibers and bacterial contamination in the fibers.

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ACKNOWLEDGMENTS

The author acknowledges support by NIH/NIDDK.

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