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In Vitro and In Vivo Stability of Electrode Potentials in Needle-Type Glucose Sensors

Influence of Needle Material

G. VELHO, P. FROGUEL, R. STERNBERG, D.R. THEVENOT, AND G. REACH

Enzymatic glucose sensors are based on the amperometric detection of an oxidable species generated during the oxydation of glucose by glucose oxidase. This measurement usually requires a working electrode (anode), an auxiliary electrode (cathode), and a reference electrode, the function of the latter being to keep constant the working potential of the anode, which is responsible for current generation. However, in the needle-type glucose sensors proposed so far, the reference electrode is missing, and its function is performed by the auxiliary electrode. We investigated, in vitro and in vivo in rats, the ability of several cathode-needle materials to behave as a reference electrode in two-electrode glucose sensors, i.e., to present a stable auxiliary electrode potential. In vitro, when glucose concentration was raised from 0 to 30 mM, the auxiliary potential of both gold- and silver-coated sensors presented a cathodic drift, whereas that of silver/silver chloride–coated sensors remained stable. In vivo, during insulin-induced hypoglycemia (5.9–2.4 mM), the auxiliary potentials of all sensors remained stable, whereas during glucose infusion (mean blood glucose concentration 11.2 mM), the auxiliary potentials of both gold- and silver-coated sensors presented an anodic drift, whereas those of silver/silver chloride–coated sensors remained stable. We also indirectly quantified the changes in sensor response induced by variations in the working potential in vitro and in vivo, simulating those that might be produced by a drift in the auxiliary potential. Such changes in the working potential could bring about a 30% unspecific variation in sensor response. We conclude that improvements in sensor analytical characteristics should be obtained with silver/silver-chloride–coated cathodes. *Diabetes* 38:164–71, 1989

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Enzymatic glucose sensors, used as part of a closed-loop insulin-delivery system, consist of electrodes associated to glucose oxidase (GOD). During oxidation of glucose by the enzyme, an electrochemically oxidable molecule, such as hydrogen peroxide, is generated and can be detected amperometrically as a current by the electrodes (1). The usual three-electrode setup for the amperometric detection of any oxidable species (2), particularly hydrogen peroxide, is composed of a working electrode (anode), an auxiliary electrode (cathode), and a reference electrode connected to an amperometric detector (Fig. 1). When an adequate voltage difference is applied between the working and auxiliary electrodes (Va − Vd), hydrogen peroxide is oxidized on the anodic surface of the working electrode, generating an electric current that can be measured. This voltage difference between the working and the auxiliary electrodes can be decomposed in three components from the equation Va − Vd = (Va − Vb) + (Vd − Vc) + (Vb − Vc). The first two terms represent, respectively, the working and auxiliary potentials, i.e., the voltage difference between the working or auxiliary electrode and the sample solution in its vicinity; the third term corresponds to the drop in potential within the solution between the vicinities of the working and the auxiliary electrodes. The latter component can be neglected; it would not be greater than a few millivolts, due to the very low values of output current (in the nA range) generated in this kind of system and to the high concentration of electrolytes in the surrounding milieu.

The intensity of the current, generated by the sensor in response to hydrogen peroxide, is proportional to the hydrogen peroxide concentration in the bulk solution. It also depends on the magnitude of Va − Vb. Thus, this potential should be kept constant for the optimal functioning of the system, because variations would produce unspecific changes in the sensor signal. However, auxiliary electrodes, because of the nature of the material they are made of, might

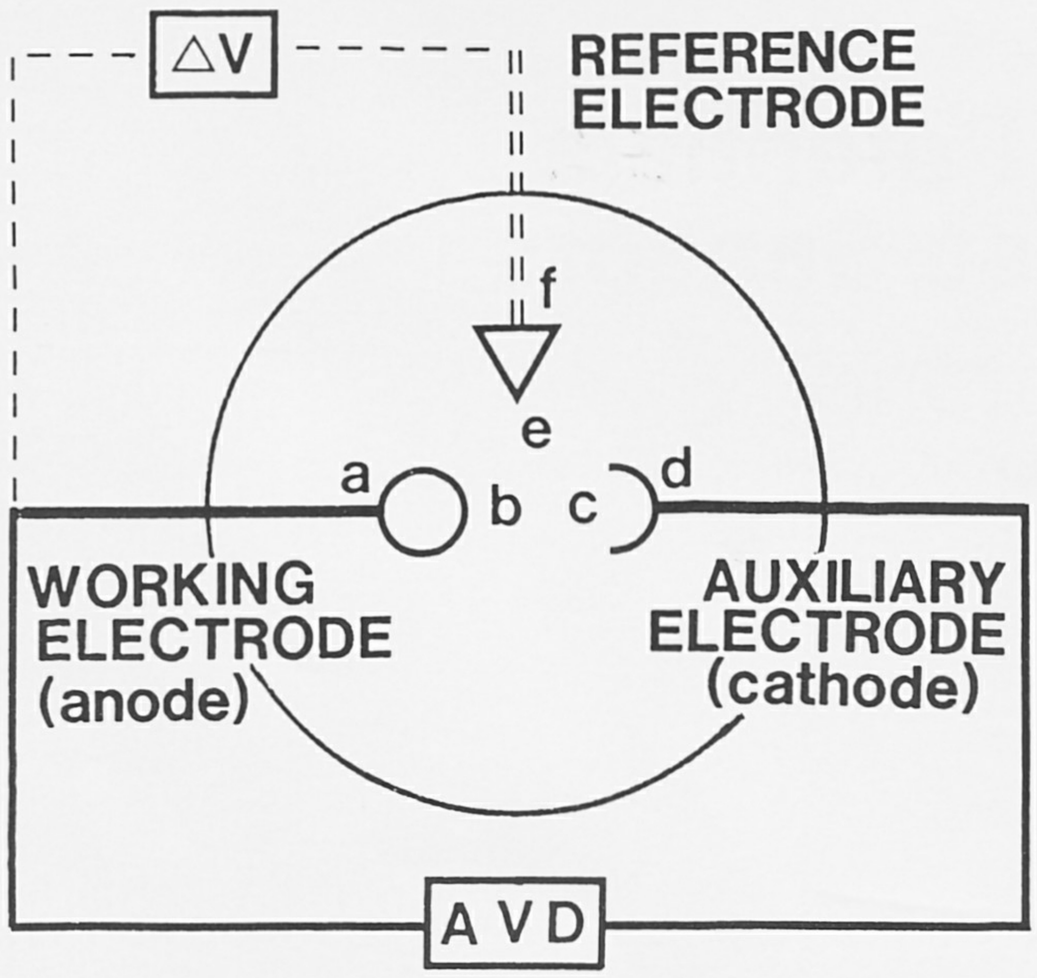

FIG. 1. Schema of amperometric system for hydrogen peroxide detection. Working potential (Va − Vb), responsible for current generation, results from voltage difference (AVD) applied between working and auxiliary electrodes (Va − Vd), expressed as algebraic difference between this applied potential and auxiliary potential (Vd − Vc). In 3-electrode setup, measurement of AVD between reference and working electrodes (Vf − Va) and its comparison to a preset value allows for correction of any drift in working potential by adjustment in AVD. In 2-electrode setup, working potential may drift unchecked during variations of Vd − Vc.

not always behave as an electrochemical reference system; indeed, as current flows through them, Vd − Vc may vary. If Va − Vd is maintained constant, these variations of Vd − Vc would, in turn, result in changes in Va − Vb. This is why a third electrode, serving as a reference, is introduced to the setup to control the stability of the working potential. This is made possible by the continuous measurement of Vf − Va and its comparison to a preset value. Any drift from this preset value is immediately corrected by the amperometric unit, which applies a correcting voltage difference between working and auxiliary electrodes.

Needle-type enzymatic glucose sensors, associating GOD with a hydrogen peroxide detector, have been proposed for subcutaneous implantation as part of a closed-loop insulin-delivery system (3–5). However, the electrochemical detector of these sensors presents an unusual configuration; it features only two electrodes, the anodic working electrode and a cathodic auxiliary reference electrode, which actually works as a pseudoreference (Fig. 1). Such electrodes are indeed made of nonoxidized silver (3,4) or even stainless steel (5), which are not known to be reference-electrode materials. Therefore, as current flows through such a pseudo–reference electrode, its potential, i.e., the voltage difference measured between the electrode and the bulk solution (Vd − Vc), may be related to this current, the sensor response itself. Thus, in these sensors, the effective working potential, responsible for current generation, is not well defined and results from the algebraic difference between two components: the fixed-applied voltage between the working and the pseudo–reference electrodes (Va − Vd) and the variable pseudo–reference potential (Vd − Vc). Thus, the value of the effective working potential may drift in response to variations of this latter component.

The aim of this work was therefore to evaluate the ability of different cathode-needle materials, the auxiliary/reference electrode of two-electrode glucose sensors, to perform as stable reference electrodes. We compared the analytical patterns of needle-type glucose sensors that had a working anode made of platinum and an auxiliary/reference cathode made either of silver, gold, or silver/silver chloride. We measured the actual difference in voltage between the sensor's cathode-needle and a true micro–reference electrode and studied its variations in response to changes in glucose concentration. This difference in voltage, which represents an indirect assessment of the pseudo–reference potential (Vd − Vc, Fig. 1), will be referred to as *cathode/reference voltage difference*. To quantify the influence of the changes in the pseudo–reference potential on the sensors' response, we also studied the effects of variations in applied voltage difference on the sensors' output current. The rationale of the latter experiments was that variations in applied voltage difference would bring about much the same changes on the working potential as those caused by variations in pseudo–reference potential. These studies were carried out in vitro and in vivo with sensors implanted in the subcutaneous tissue of anesthetized rats.

MATERIALS AND METHODS

Needle-type glucose sensor and reference electrode. The design of the sensor was modified from Shichiri et al. (3). Briefly, the cathode was made of a steel needle (23 G) coated with gold (5-μm layer; $n = 4$ in vitro, $n = 3$ in vivo), silver (15-μm layer; $n = 5$ in vitro, $n = 3$ in vivo), or silver/silver chloride (15-μm layer; $n = 5$ in vitro, $n = 3$ in vivo) by electrolysis (Usiflamme, Saint Gratien, France). The anode was formed by the tip of a 0.2-mm platinum wire, melted by a gas flame to form a microsphere (~1 mm diam), then electrically isolated inside a polyethylene catheter (Biotrol, Paris) and cast in an epoxy resin inside the cathode needle. The tip of the electrode was then dipped in a suspension of 2000 U/ml glucose oxidase (grade II, 300 U/mg, Boehringer Mannheim, Mannheim, FRG) and 1000 U/ml heparin (H-108, Choay, Paris) in acetone and ethanol (1:1 vol) containing 2.5% cellulose acetate (Prolabo, Paris). After air drying for 24 h, the electrode was dipped in a 1% glutaraldehyde aqueous solution (Prolabo) and again air-dried for 24 h. It was then dipped in a solution of 5% polyurethane (EG85A, Thirmedics, Woburn, MA) in dimethylformamide and tetrahydrofuran (1:9 vol, Prolabo). The needles themselves were not covered with cellulose acetate and polyurethane layers and thus were directly in contact with buffer solution or subcutaneous interstitial fluid. After preparation, all sensors were kept dry at 4°C. The in vitro characteristics of the sensors ($n = 14$; Table 1), i.e., sensitivity expressed in nanoamperes per millimolar concentration of glucose, linear range of the calibration curve, the response time (t_{90}%), and variations of the cathode-reference voltage difference were determined in a stirred, buffered solution (0.15 M phosphate, 0.15 M

TABLE 1
In vitro and in vivo characteristics of glucose sensor

	Cathode material			
	Ag	Au	Ag/AgCl	Total
In vitro				
n	5	4	5	14
ΔI/ΔC (nA/mM)	1.10 ± 0.37	4.84 ± 1.35	4.43 ± 0.92	3.96 ± 0.69
I_0 (nA)	7.76 ± 0.81	12.00 ± 2.20	9.76 ± 2.11	9.69 ± 1.12
Linearity (mM)	18.6 ± 1.7	15.5 ± 1.6	16.8 ± 0.4	17.1 ± 0.8
Temperature coefficient (%/°C)				2.86 ± 0.59
In vivo				
n	3	3	3	9
ΔI/ΔC (nA/mM)	1.15 ± 0.13	1.14 ± 0.10	2.64 ± 0.10	1.64 ± 0.24
I_0 (nA)	4.09 ± 2.00	6.66 ± 2.76	0.40 ± 0.16	3.71 ± 1.42

Sensitivity coefficients are expressed as ΔI/ΔC, background currents by I_0, linearity by upper limit of linearity range of calibration curve; temperature coefficients express percentage of variation of sensitivity coefficient per °C.

NaCl, pH 7.4) at 35–37°C during stepwise increases in the glucose concentration from 0 to 30 M.

The external micro–reference electrode was formed by a silver/silver-chloride wire (0.4 mm diam) placed inside a Teflon catheter (1 mm diam, 4 cm length) containing physiological saline (0.15 M NaCl) and sealed by an ion-permeable diaphragm.

In vitro studies. The two-electrode sensor was connected to an amperometric unit (PRG-Del, Solea Tacussel Electronique, Villeurbanne, France) applying a 650-mV charge between the platinum anode and the cathode needle and allowing for current measurement in nanoamperes. The sensor's cathode and the external reference electrode were connected to a high-impedance voltmeter (S6N, Solea Tacussel Electronique), allowing for measurement of voltage difference in millivolts. Both signals were recorded by a two-channel penrecorder (SE 120, Goerz Electro, Vienna, Austria).

The influence of variations of the pseudo–reference potential on the generated current was indirectly assessed by determining the changes in the sensor output current at 6 mM glucose in response to 10-, 50-, and 100-mV increments or decrements in the applied voltage difference between the sensor's anode and cathode.

In vivo studies. Overnight fasted male Wistar rats (n = 9, 250–300 g, Janvier, Saint Berthevi, France) were anesthetized with pentobarbital sodium (50 mg/kg i.p., Clin Midy, Saint Jean de la Ruelle, France), and polyethylene (Biotrol 3, Biotrol, France) and silicone (Silastic 602-135, Dow Corning, Midland, MI) catheters were inserted into the left jugular vein and homolateral carotid artery, respectively, for glucose or insulin injection and for blood sampling for the determination of blood glucose concentration. After the in vitro characterization, the sensor and the reference electrode were implanted in the subcutaneous tissue through a small cervical incision. Animals were kept spontaneously breathing under a flow of carbogen (95% O_2/5% CO_2) and were warmed under a lamp to prevent peripheral vasoconstriction.

The in vivo characteristics of the output current and the cathode-reference voltage difference were determined during variations in subcutaneous glucose levels induced by insulin injection and glucose infusion (*n* = 9; Table 1). At least 1 h after surgery, when blood glucose and both electrodes' outputs were stable, 1 U of insulin (Endopancrine U40, Organon, Saint Denis, France) was injected through the jugular catheter. When the insulin-induced fall in blood glucose reached a plateau, a continuous intravenous glucose infusion (20–60 mg · kg^{-1} · min^{-1}; infusion pump Infu 362, Datex, Uhwiesen, Switzerland) was begun to bring blood glucose and subcutaneous glucose to hyperglycemic levels. Blood samples (0.3 ml) were serially drawn through the carotid catheter at 5-min intervals, −10 to 50 min during insulin-induced hypoglycemia, 0 to 40 min during glucose infusion. Samples were immediately centrifuged and plasma glucose concentration was determined with a glucose analyzer (Beckman, Fullerton, CA). An in vivo method for sensor calibration was used to calculate the apparent subcutaneous glucose level (6). Briefly, after insulin injection, the sensor output decreased to a plateau, as did the blood glucose concentration. The preinsulin and postinsulin steady states of the blood glucose level and the sensor output made it possible to calculate, by a two-point calibration of the sensor, both the in vivo sensitivity coefficient, expressed in nanoamperes per millimolar concentration, and the in vivo background current, i.e., the extrapolated signal corresponding to 0 mM glucose (I_0). The estimated subcutaneous glucose was obtained by subtracting the in vivo background current (I_0) from the sensor current observed at a given time and then dividing the result by the sensitivity coefficient.

The influence of variations in the pseudo–reference potential on the generated current was assessed indirectly by determining the changes in the sensor output current at fasting glucose level (6.1 ± 0.1 mM, *n* = 6) in response to 10-, 50-, and 100-mV increments or decrements in the applied voltage difference between the sensor's anode and cathode.

Expression of results and statistics. All data in text and figures are given as means ± SE, and their statistical significance was assessed by the two-tailed paired *t* test (7).

RESULTS

In vitro determination of cathode-reference voltage difference. The variations of the sensor output current and the cathode-reference voltage difference during stepwise 5 mM increases in glucose concentration are shown in Fig. 2. Up to 20 mM, the sensor output current increased linearly from

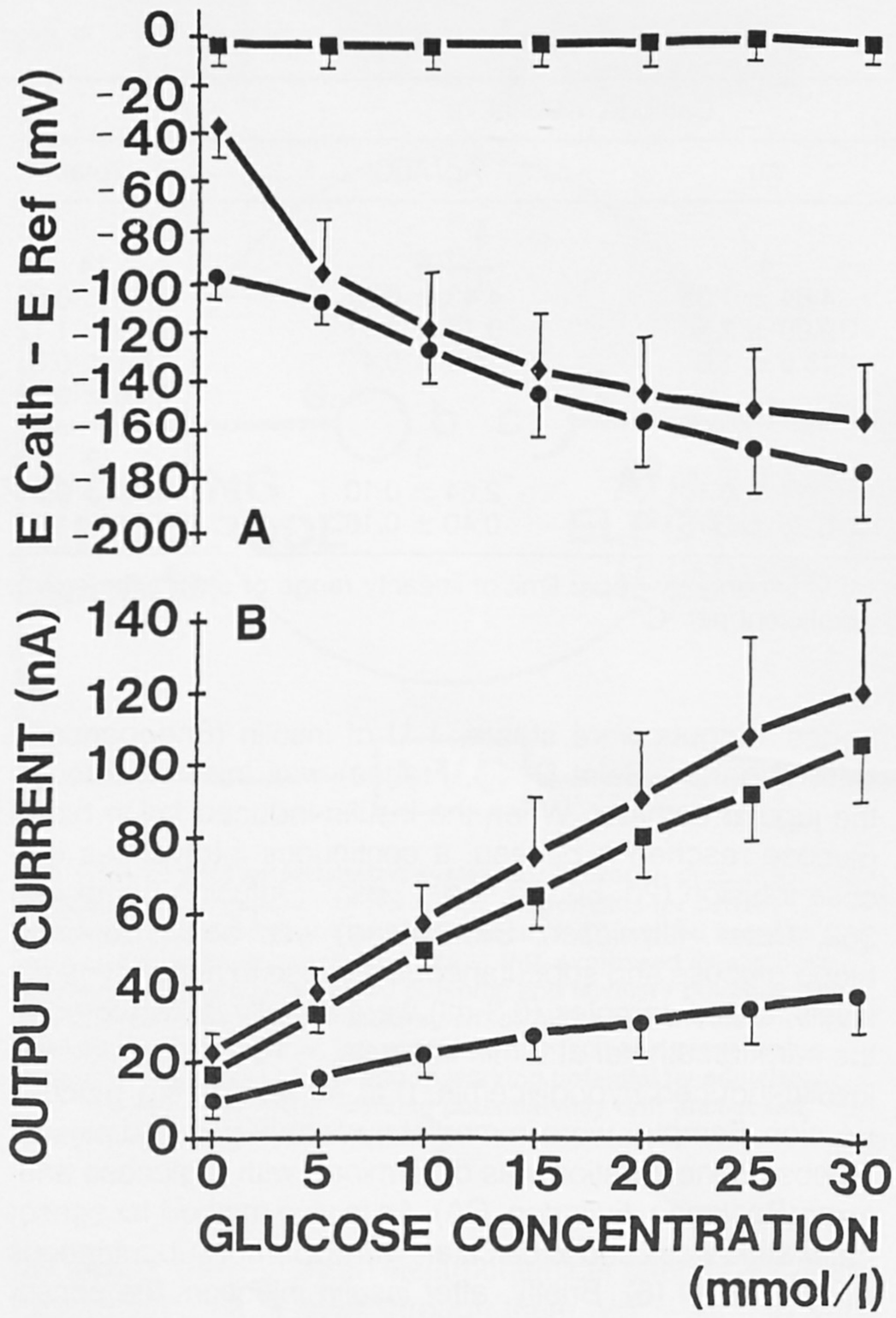

FIG. 2. In vitro variations of needle cathode–reference electrode voltage difference (*A*) and sensor output current (*B*) during stepwise 5-mM increases in glucose concentration. Results are means ± SE. ●, Silver-coated sensors (n = 5); ♦, gold-coated sensors (n = 4); ■, silver/silver chloride–coated sensors (n = 5).

3.54 ± 0.52 to 23.18 ± 7.12 nA for the silver-coated sensors (n = 5), from 5.08 ± 0.75 to 77.80 ± 20.80 nA for the gold-coated sensors (n = 4), and from 5.76 ± 1.61 to 68.16 ± 9.68 nA for the silver/silver chloride–coated sensors (n = 5). Within this linearity range, the cathode-reference voltage difference decreased from −95 ± 7 to −154 ± 19 mV (n = 5, $P < .05$) for silver-coated sensors, and from −37 ± 13 to −145 ± 26 mV (n = 4, $P < .01$) for gold-coated sensors. In contrast, it remained stable at −4 ± 7 mV (n = 5, NS) for silver/silver chloride sensors.

In vitro determination of cathode-reference voltage difference. After the intravenous injection of 1 U insulin, blood glucose level decreased from 5.9 ± 0.1 to a 2.4 ± 0.2 mM plateau (n = 9) (Fig. 3). The output current generated by the sensor decreased from 10.96 ± 1.27 to 6.45 ± 1.95 nA (n = 3) for silver-coated sensors, from 13.22 ± 3.29 to 9.6 ± 2.94 nA (n = 3) for gold-coated sensors, and from 14.48 ± 0.70 to 5.17 ± 0.34 nA (n = 3) for silver/silver chloride–coated sensors. The resulting in vivo characteristics of the sensors are summarized in Table 1. The apparent subcutaneous glucose concentration, calculated from the sensor output and taking into account the in vivo sensitivity coefficient of each experiment, followed blood glucose level with a 5-min lag. Note that after this transformation of the data, the estimation of the apparent subcutaneous glucose concentration was highly reproducible, as indicated by the small SE. The large SE of the output current was due only to differences in the sensitivities of the sensors (6). Table 2 shows the data for the individual sensors.

The cathode-reference voltage difference presented no significant variations: from −253 ± 16 to −249 ± 17 mV (n = 3, NS) for silver-coated sensors, from −261 ± 32 to −255 ± 30 mV (n = 3, NS) for gold-coated sensors, and from −181 ± 45 to −177 ± 43 mV (n = 3, NS) for silver/silver chloride–coated sensors. The large values of the SE

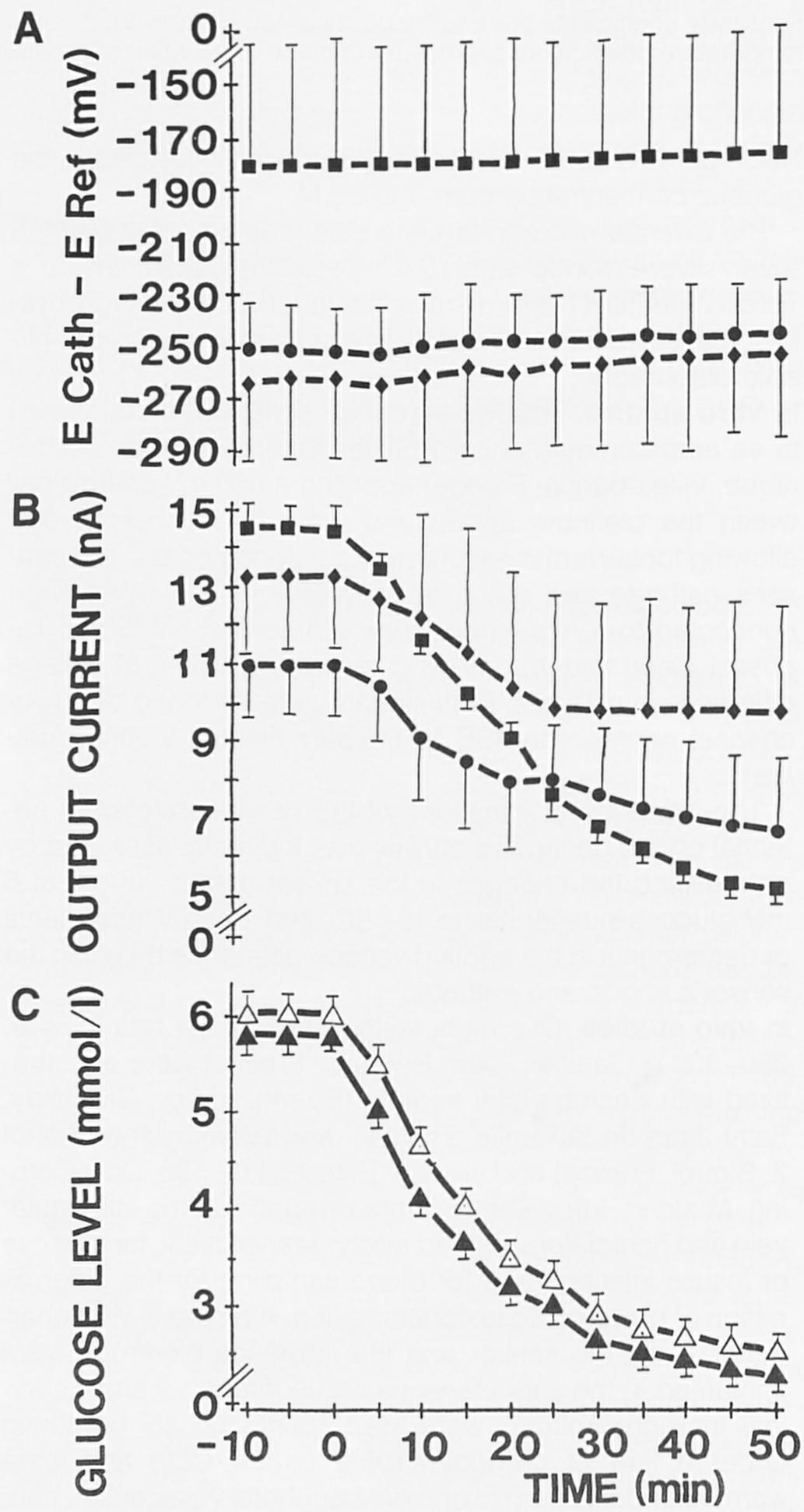

FIG. 3. In vivo variations of needle cathode/reference electrode voltage difference (*A*) and sensor output current (*B*) during insulin-induced hypoglycemia. Results are means ± SE (n = 3 for each type of sensor). ●, Silver-coated sensors; ♦, gold-coated sensors; ■, silver/silver chloride–coated sensors. *C*: blood glucose (n = 9; ▲) and calculated subcutaneous glucose concentration (△).

TABLE 2
In vivo sensor response and cathode potential for individual sensors during insulin-induced hypoglycemia and glucose infusion

	Insulin injection						Glucose infusion					
	In vivo sensor response (nA)			Cathode potential (mV)			In vivo sensor response (nA)			Cathode potential (mV)		
Sensor	t_0	t_{50}	ΔI	t_0	t_{50}	ΔE	t_0	t_{40}	ΔI	t_0	t_{40}	ΔE
Ag 1	8.64	3.04	−5.60	−284	−280	4	3.20	11.84	8.64	−278	−228	50
Ag 2	13.92	11.04	−2.88	−258	−258	0	10.40	17.20	6.80	−258	−226	32
Ag 3	10.16	5.28	−4.88	−216	−208	8	5.20	21.04	15.84	−206	−168	38
Au 1	9.60	7.68	−1.92	−310	−304	6	7.44	13.36	5.92	−306	−264	42
Au 2	21.20	16.56	−4.64	−184	−184	0	17.36	27.12	9.76	−184	−154	30
Au 3	8.80	4.56	−4.24	−292	−278	14	4.56	16.32	11.76	−276	−252	24
Ag/AgCl 1	16.24	5.28	−10.66	−250	−244	6	5.00	26.00	21.00	−242	−238	4
Ag/AgCl 2	13.20	4.40	−8.80	−218	−218	0	4.40	24.39	19.99	−216	−216	0
Ag/AgCl 3	14.00	5.84	−8.16	−72	−72	0	5.84	25.40	19.56	−76	−72	4

Time (t) expressed in minutes; ΔI and ΔE express difference between values of sensor response and cathode potential at end and beginning of experiment.

reflect only individual variability; the cathode-reference voltage difference of each individual sensor remained perfectly stable throughout the experiment (C.V. <1%, Table 2).

During glucose infusion (Figure 4), the blood glucose concentration increased from 2.5 ± 0.2 mM to a 11.2 ± 0.2-mM plateau at 40 min. Meanwhile, the estimated subcutaneous glucose level, averaged for all sensors, rose to a 10.6 ± 0.3-mM plateau (n = 9, NS vs. blood glucose level). The output current increased from 6.26 ± 1.75 to 16.56 ± 2.11 nA (n = 3) for silver-coated sensors, from 9.79 ± 3.17 to 18.93 ± 3.41 nA (n = 3) for gold-coated electrodes, and from 5.27 ± 0.48 to 25.27 ± 0.38 nA (n = 3) for silver/silver chloride–coated sensors. The cathode-reference voltage difference increased from −247 ± 18 to −210 ± 15 mV (n = 3, $P < .05$) for silver-coated sensors and from −255 ± 30 to − 223 ± 28 mV (n = 3, $P < .05$) for gold-coated sensors. Again, it remained stable between −178 ± 42 and −175 ± 43 mV (n = 3, NS) for silver/silver chloride–coated sensors (C.V. <1%, Table 2). Therefore, with gold- and silver-coated electrodes, this instability in the auxiliary potential might result in an increase in the working potential during glucose infusion.

Influence of variations of the applied voltage difference on sensor response. Table 3 shows the variations in the output currents observed both in vitro and in vivo when the applied voltage difference was changed from its preset value of 650 mV, either down to 550 mV or up to 750 mV. Both in vitro or in vivo, the observed current was linearly correlated to the applied voltage difference ($r > .968$ for the 4 conditions). More precisely, when the applied voltage difference was increased by 10 mV in vivo, a change which might result from the change in cathode-reference voltage difference commonly observed during glucose infusion (Fig. 4), the current increased from 12.13 ± 1.48 to 12.93 ± 1.46 nA ($P < .05$) and from 10.67 ± 0.60 to 11.53 ± 0.79 nA (P = .06) for gold- and silver/silver chloride–coated sensors, respectively. Figure 5 illustrates these variations, expressed as a percentage of the value observed at 650 mV. The influence of the change of the applied voltage difference on the current was similar for the two kinds of tested electrodes, both in vitro and in vivo.

DISCUSSION

The influence of various cathode materials on the analytical performances of needle-type glucose sensors has not been investigated, although the ability to maintain a stable working potential during hydrogen peroxide oxidation may be of paramount importance for the functioning of the sensor. Furthermore, although it is usual in most amperometric detection systems to use either a three-electrode setup or a two-electrode system containing a large area reference-counter electrode, as in the Clark-type oxygen sensor (8), the glucose sensors developed up to now have only two electrodes, neither of which is a reference electrode. In systems containing a real reference electrode, the working potential is constant and the output current is proportional to the bulk concentration of analyte. In contrast, when slow electrochemical reactions such as hydrogen-peroxide oxidation are performed in systems whose working potential may vary with time, current intensity, or composition of the fluid surrounding the electrodes, as in sensors not having a reliable reference electrode, several abnormal analytic behaviors may be detected: nonlinearity, irreproducibility within one sensor, and irreproducibility from one sensor to another.

We compared various two-electrode glucose sensors with a platinum working electrode as anode and either a silver, gold, or silver/silver chloride–coated auxiliary electrode as cathode to determine whether these different cathode materials could behave appropriately as reference electrodes, that is, allow the maintenance of a stable working potential. This working potential, in such two-electrode sensors, is the algebraic difference between the voltage difference applied between the working and the auxiliary electrodes (Va − Vd, Fig. 1) and the pseudo–reference potential itself (Vd − Vc). It is the behavior of the latter potential that was indirectly assessed during glucose detection. It was measured as the voltage difference between the cathode needle and a true reference electrode and described as cathode-reference voltage difference. When we compared the glucose sensors presenting various needle materials, we took into account two parameters: the actual value of the cathode-reference voltage difference vs. the real reference electrode and the drift of this cathode potential when the sensor output current

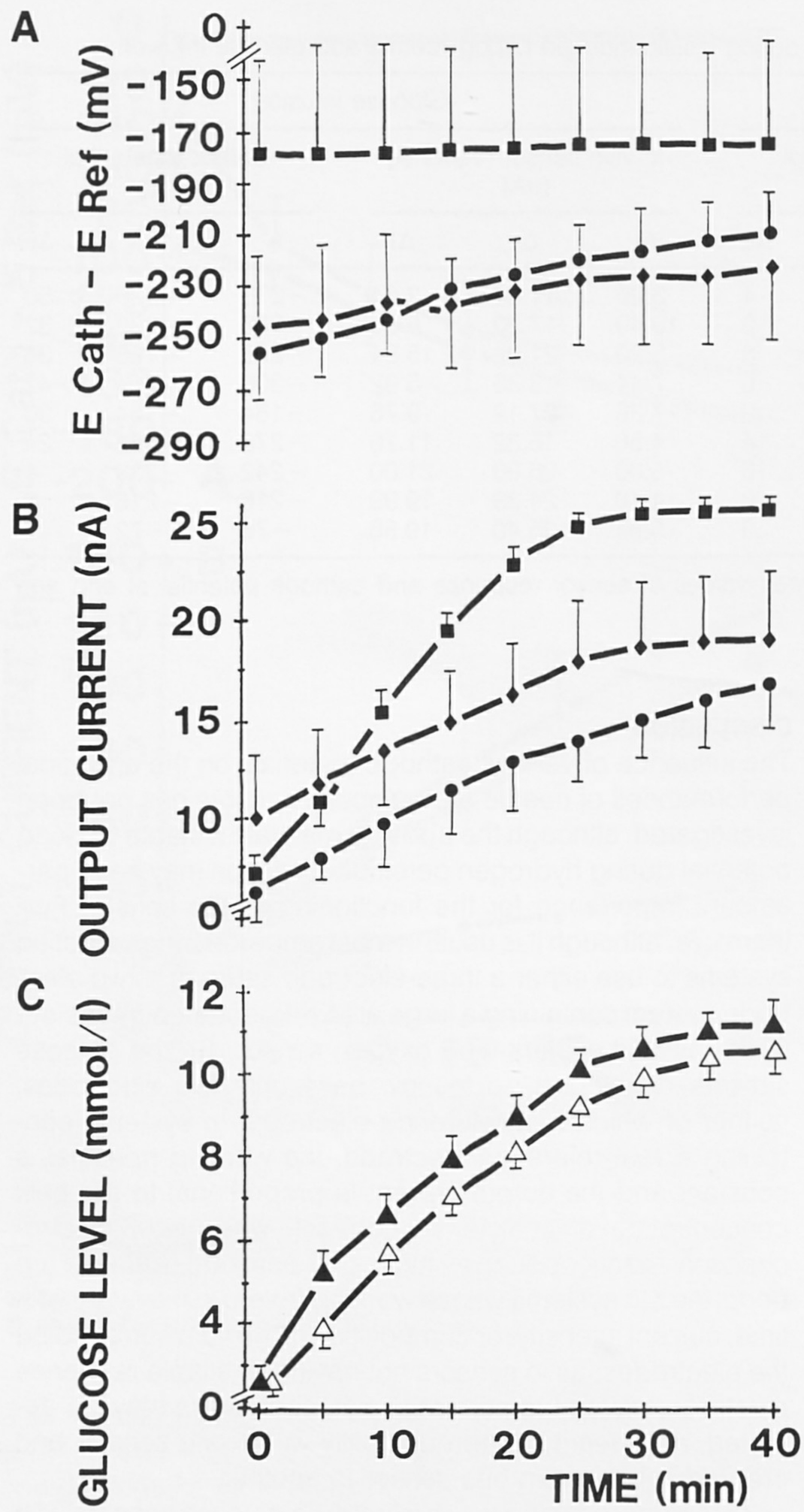

FIG. 4. In vivo variations of needle cathode–reference electrode voltage difference (*A*) and sensor output current (*B*) during glucose infusion. Results are means ± SE (*n* = 3 for each type of sensor). ●, Silver-coated sensors; ♦, gold-coated sensors; ■, silver/silver chloride–coated sensors. *C*: blood glucose (*n* = 9; ▲) and calculated subcutaneous glucose concentration (△).

changes in response to variations in glucose concentration. For the sake of clarity, we independently determined the sensor output current and its corresponding cathode-reference voltage difference.

Such a procedure gave important but not surprising results when sensors were tested in vitro (Fig. 2): silver/silver chloride–coated needles presented a negligible average cathode-reference voltage difference, −4 mV, compared with −37 and −95 mV, respectively, for gold- and silver-coated needles. Thus, in the case of silver/silver chloride sensors, the effective working potential was virtually equal to the applied potential. Differences of cathode potential among the three types of sensors could account for the differences in their sensitivity coefficients (Table 1), although we cannot exclude the possibility that the latter results are in part due to the irreproducibility of the enzymatic and/or polyurethane layer deposition procedures. Silver/silver chloride needles showed no change in cathode potential when the glucose concentration was progressively raised to 20 mM. Silver- and gold-plated needles fared less well, with an average potential drift of 59 and 108 mV, respectively, toward more cathodic potentials. Similar results were obtained with H_2O_2, up to 12 μM instead of glucose (data not shown). When the sensors were assessed in a buffered solution containing physiological concentrations of phosphate, sodium chloride, protein, bicarbonate, and potassium, comparable results were observed: a stability of the cathode-reference voltage difference for the silver/silver chloride needles and a cathodic drift for the gold needles (*n* = 3 each; data not shown). These results are not surprising: there is no electrochemical reason to think that the addition of these substances to the buffer would make a gold electrode stable in potential. Nevertheless, the results rule out these substances as the cause of the different behaviors of the cathode potential in vitro and in vivo.

More unexpected results were obtained with in vivo assessments; although the potentials of gold- and silver-coated needles were more cathodic than the potential of silver/silver chloride–coated needles, all types, including the latter, were much more cathodic than expected. Indeed, as shown in Fig. 3, whatever the glucose concentration and the current value, a −175 ± 45 mV (*n* = 3) difference was observed between the silver/silver chloride–coated needle and the external reference microelectrode (Ag/AgCl, 0.15 M NaCl) implanted in the subcutaneous tissue, in which the chloride concentration is probably also 0.15 M. As we have cautiously checked the electrical independence of the potentiostat and the high-impedance millivoltmeter units, such large differences in potential may be related to the distance between the implantation sites on both electrodes, 0.5–1.0 cm, and the development of local potential difference within the cervical region of the animal. Another surprising result was obtained when comparing cathode-reference voltage difference drifts during insulin-induced hypoglycemia (Fig. 3) and glucose-infusion–induced hyperglycemia (Fig. 4): whereas in the former situation all types of sensors showed no significant drift in potential, in the latter case an average anodic drift of 32 and 37 mV was observed when blood glucose concentration increased from 2.5 to 11.2 mM and the output current consecutively increased from 9.8 to 18.9 nA and from 6.2 to 16.5 nA for the gold- and silver-coated sensors, respectively. Here again, the cathode potential of silver/silver chloride sensors remained remarkably constant. The nature of the cathode-potential controlling factor, responsible for the unusual anodic shift of gold and silver sensors during glucose infusion, was not determined.

Because the plasma level of potassium, sodium, and phosphate presented only minimal changes during experiments of insulin-induced hypoglycemia and glucose infusion (data not shown), it is tempting to relate this anodic drift to the generation of some other electroreducible species during intravenous administration of glucose. This species may

TABLE 3
Dependence of in vitro and in vivo sensor output current on applied voltage difference between platinum anode and gold or silver/silver chloride cathode

	In vitro		In vivo	
Applied voltage difference (mV)	Au ΔI (nA)	Ag/AgCl ΔI (nA)	Au I_0 + ΔI (nA)	Ag/AgCl I_0 + ΔI (nA)
550	8.87 ± 1.49	7.16 ± 1.54	8.47 ± 1.19	7.67 ± 0.33
600	9.47 ± 1.46	7.51 ± 1.53	10.13 ± 1.57	8.87 ± 0.36
640	10.07 ± 1.56	7.87 ± 1.46	11.33 ± 1.48	10.13 ± 0.54
650	10.33 ± 1.50	8.05 ± 1.47	12.13 ± 1.48	10.67 ± 0.60
660	10.93 ± 1.50	8.36 ± 1.49	12.93 ± 1.46	11.53 ± 0.79
700	11.93 ± 1.66	9.21 ± 1.44	15.60 ± 2.10	14.20 ± 1.34
750	13.20 ± 1.99	10.09 ± 1.39	18.67 ± 3.60	17.47 ± 2.88

Values are means ± SE; n = 3. In vitro, ΔI corresponds to 0–6 mM glucose concentration increase; in vivo, sensor output (I_0 + ΔI) corresponds to fasting glucose level (6.1 ± 0.1 mM, n = 6). Correlation between applied voltage difference and sensor response, r > .96 for all 4 cases.

be different from glucose itself, because, interestingly, when tested in vitro the increase in glucose concentration elicited on the contrary a cathodic drift. Note also that the insulin bolus did not elicit any perceptible change in the cathode potential of silver or gold sensors as well, and this could be related to the very different physiological modifications yielded by insulin bolus and glucose infusion. Further in vivo experiments were not performed to determine the origin of this anodic drift observed during glucose infusion. Whatever the nature of the responsible species, the fundamental cause of these in vitro and in vivo drifts of cathode-reference voltage difference is the ill-defined cathode potential of gold or silver cathodes. In fact, these cathodes cannot, on solid electrochemical basis (and on the experimental data shown in this article), be as stable in potential as silver/silver chloride cathodes are (9). Thus, electrochemical systems using gold or silver cathodes require a third, true reference electrode to work properly.

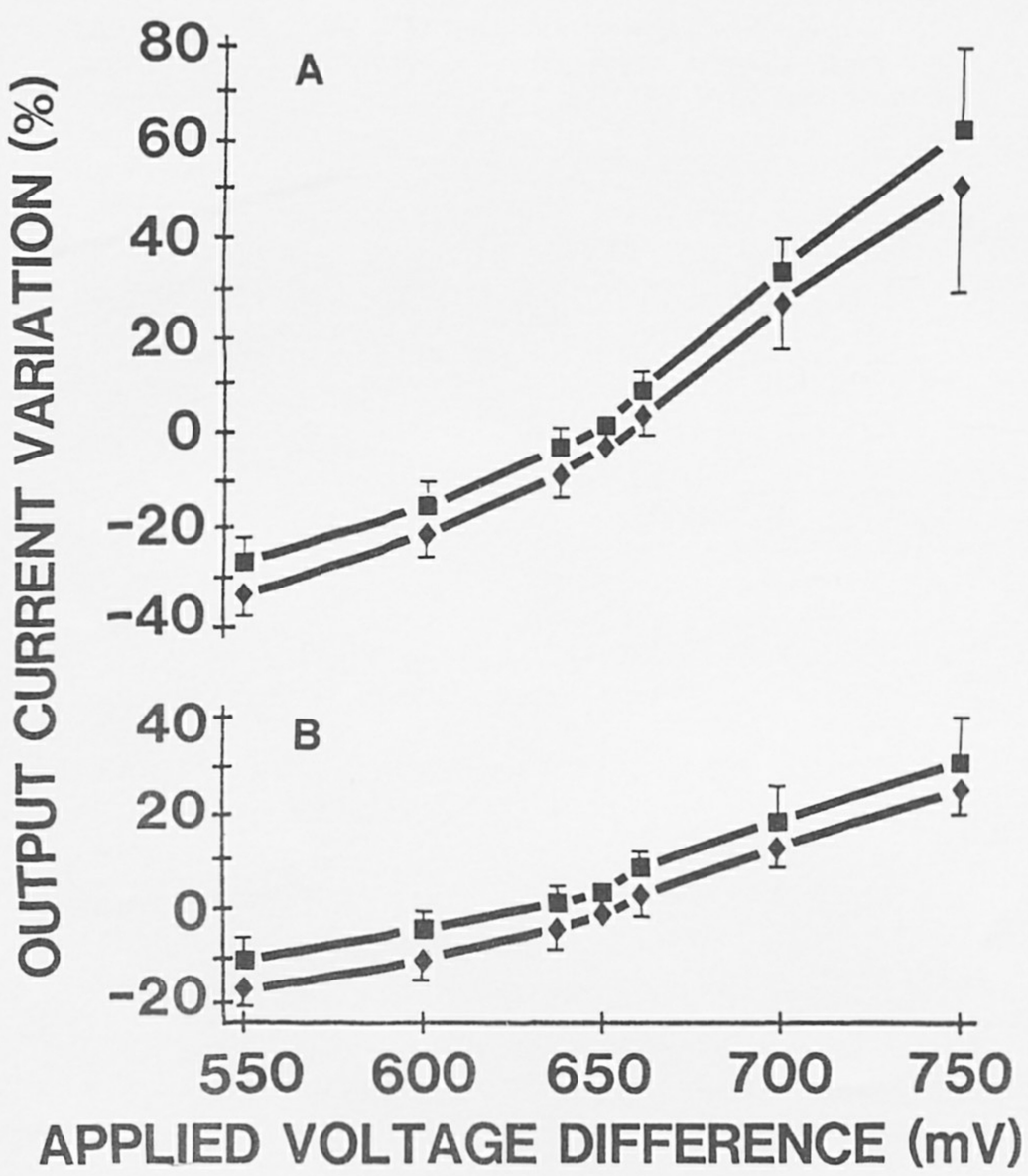

FIG. 5. Variations in sensor output during increase and decrease of voltage difference applied between platinum anode and cathode needle from its 650-mV base value. In vivo results (*A*) are expressed as percentage change of sensor output current (I_0 + ΔI) corresponding to fasting glucose level (6.1 mM glucose). In vitro results (*B*) are expressed as percentage change of sensor response (ΔI) corresponding to 0–6 mM glucose variation. ♦, Gold-coated sensors; ■, silver/silver chloride–coated sensors. Data are means ± SE (n = 3 for each type of sensor).

The point that should be stressed is that the cathode-reference voltage difference of silver/silver chloride sensors remained remarkably stable, in vitro and in vivo, during upward and downward changes in glucose level and was insensitive to electroreducible species generated by insulin-induced hypoglycemia or glucose infusion. In fact, silver/silver chloride electrodes are well known to present stable potentials in amperometric environments (10). For this reason, they have been used extensively in other areas of biology and medicine, in vitro and in vivo, as auxiliary and/or reference electrodes, for the measurement of dissolved oxygen (8), pH (11), ions (12), glucose (13), and other species (14). Recently, two groups developing needle-type subcutaneous glucose sensors (15,16) proposed silver/silver chloride electrocardiogram external reference electrodes as an alternative to implanted auxiliary/reference electrodes.

The final part of this work demonstrates both in vitro and in vivo the importance of a stable working potential and quantifies the effects of its eventual drifts on current generation. Indeed, hydrogen peroxide anodic oxidation rate has been found to depend strongly on working electrode potential as indirectly demonstrated in this work by the important influence of the applied potential on the sensor response. Both silver/silver chloride and gold coatings gave similar results, both in vitro and in vivo, because actually plotted by this method was steady-state current versus potential curves, which involve hydrogen peroxide oxidation at the anodic surface, i.e., a phenomenon independent of the nature of the cathode. Thus, an increase of 50 mV in the working potential, as it could result from the increase in the cathode potential seen during glucose infusion, yields a 30% increase in the in vivo sensitivity coefficient and should not be considered a negligible artifact.

In conclusion, this study presents evidence of a strong dependence of two-electrode needle-type sensor performances, such as sensitivity, linearity, and reproducibility, on

the pseudo–reference (cathode) potential. This potential is shown to behave differently during glucose detection, depending on the nature of the cathode material. Low sensitivity or linear range of glucose sensors are not related to the use of two-electrode setup but may be related only to the use of a cathode playing the role of both auxiliary and reference electrode with a poor control of its potential. We show, by varying the applied potential difference, that sensitivity and linearity modifications are indeed related to such potential drifts.

Thus, although some results that seem clinically acceptable can be obtained with cathodes with such poor control of pseudo–reference potential as those made of gold, unoxidized silver, or even stainless steel, real improvement of sensor analytical characteristics should be obtained with relatively large area silver/silver chloride–coated cathodes.

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