Reverse Iontophoresis: Noninvasive Glucose Monitoring *in Vivo* in Humans

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Purpose: To demonstrate that "reverse iontophoresis" can be used to noninvasively obtain information about systemic glucose levels *in vivo* in humans.

Methods. The passage of current across the skin *in vivo* drives ions into the tissue, from the electrode chambers positioned on the skin surface, and simultaneously pulls ions from the body in the opposite direction. Because of the net negative charge on the skin, under normal conditions, the membrane is permselective to cations, and a potential gradient also results, therefore, in electroosmotic convection of solvent in the direction of counterion flow (i.e., from anode to cathode). Thus, it is also possible to enhance the transport of polar, yet uncharged, species using iontophoresis. In an earlier study, the *in vitro* extraction of glucose, by "reverse iontophoresis" was established, and extension of the approach to an *in vivo* model was indicated. The idea has therefore been further explored *in vivo* in humans.

Results. Using small, simple, prototypical electrode chambers, attached to the ventral forearm surface, direct current iontophoresis at 0.25 mA/cm^2 for periods of up to 1 hour, and a sensitive analytical procedure to measure the quantities of glucose extracted, it has been shown that iontophoretic sampling of glucose is feasible. However, the shorter periods (15 minutes or less) of extraction considered yield results which are "contaminated" (it is believed) by glucose that is a product of lipid metabolism within the skin. While this material is expected to complicate the initial calibration of the approach, the problem is effectively resolved within one hour, by which time the glucose arriving in the electrode chambers on the skin surface is expected to directly reflect the subcutaneous tissue concentration.

Conclusions. Based upon these initial observations, further investigation can now be directed towards optimization of electroosmotic flow and sampling time, improved reproducibility and the development of a practical assay methodology.

KEY WORDS: iontophoresis; "reverse iontophoresis"; electroosmosis; glucose monitoring; skin permeation.

INTRODUCTION

The currently used methods for blood glucose monitoring are invasive and subject to poor patient compliance. There is, however, very strong evidence to support the fact that the morbidity associated with diabetes mellitus can be significantly mitigated by frequent glucose monitoring and the consequently tighter control of blood sugar [1]. It is the objective of the research described here to develop a noninvasive glucose sensing technology based upon the "reverse iontophoretic" extraction (and subsequent analysis) of samples across the skin [2,3]. Iontophoresis employs the passage of a small current to promote the penetration of (typically) ionized molecules across (usually) the skin [4]. However, application of a potential difference across the skin also enhances the transport of neutral molecules [5]. The iontophoretic enhancement of uncharged species results from current-induced solvent flow (i.e., as electrostatically driven ions move through the medium, there is momentum transfer to adjacent solvent molecules, inducing flow in the latter). Neutral solute transport is thus enhanced by the convective action of the solvent. The skin, at physiological pH, has an overall negative charge and is cation-permselective, therefore. Consequently, in iontophoresis, solvent flow moves preferentially from anode to cathode [4]; hence, the feasibility of reverse iontophoretic glucose extraction and monitoring should be demonstrable most effectively at the cathode. Confirmation of this hypothesis (that the amount sampled at the skin surface is proportional to the subdermally circulating level) has been demonstrated unequivocally *in vitro* [6].

In this paper, we examine the feasibility of iontophoretic glucose monitoring *in vivo*, in man. Crucial issues to be considered are: (a) the reproducibility of the extraction process; (b) the time and current necessary to extract a measurable amount of glucose; (c) sources of measurement artifact; and (d) the degree of correlation between blood glucose levels and the amounts of substrate extracted. Here, we address the first three of these important questions.

EXPERIMENTAL

1. *Subjects.* All experiments were conducted in normal, healthy volunteers, from whom informed consent had been obtained. Measurements were made on the ventral forearm.

2. *"Extraction" Chambers.* The initial cell design for the *in vivo*, reverse iontophoresis experiments consisted of two glass electrode chambers into each of which a silver—silver chloride (Ag/AgCl) electrode was inserted through a plastic cover (Figure 1(a)). The cells were adhered to the skin via a coating of silicone grease applied to the base of the chamber. The cells were filled with electrolyte (0.55 mL) through a small port drilled in the plastic cover. The chambers were positioned approximately 8 cm apart and were secured with adhesive tape. The cell area was 1 cm^2.

Subsequently, the efficiency of the extraction process was increased significantly using a modified cell design in which the surface area-to-volume ratio of the extraction chamber was increased by an order of magnitude. The collection chambers were thin, flexible, plastic devices of area 10 cm^2, but which continued to hold a volume of only 0.55 mL (Figure 1(b)). The assembly of the cell involved adhesion of an annulus of double-sided tape to the skin. A sheet of transparent, impermeable, polyethylene film was laid over

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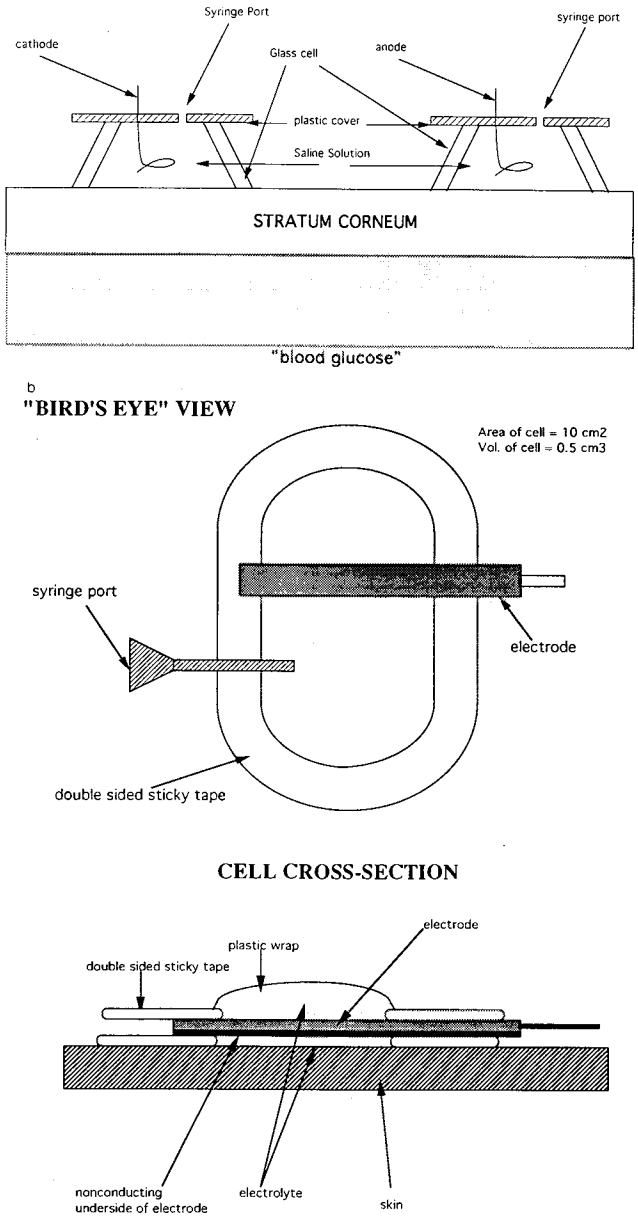

Fig. 1. (a) Schematic diagram of the *in vivo* reverse iontophoretic system used in the initial experiments. The internal diameter of the individual cells is 1 cm. Each chamber held 0.55 mL of saline. The cells were positioned a few centimeters apart on the ventral forearm. (b) "Bird's-eye" and cross-sectional views of the improved cell design, used in the latter *in vivo* experiments.

the upper surface of the adhesive ring creating, thereby, a sealed chamber. Insertion of a flat electrode and syringe port, prior to coverage with the plastic film, completed the cell assembly. These cells were positioned side-by-side on the ventral forearm with ~2 cm between them. The modified chambers were also used for the passive extraction experiments (in which case no electrodes were inserted into the devices). Although the area of the electrodes were smaller than the area of skin through which reverse electroosmotic extraction was performed, the fact that the resistance of the skin is so much greater than that of the background electrolyte (by ~100-fold or more) ensures that the solution-skin interface is an equipotential surface and that a constant current distribution exists, therefore, over the entire collection chamber area.

3. *Electrodes.* Ag/AgCl electrodes were used because they are capable of delivering significant currents without inducing changes in the pH of the bathing solutions. Preparation of the electrodes used in the primary cell design has been previously described [6]. In the modified collection chambers, the electrodes were flat strips of Ag foil onto which a layer of AgCl was electrochemically plated in the normal way [7]. The lower surface of the strip was covered with electrician's insulating tape to ensure that no electrical contact was possible between electrode and skin. Thus, the entire faradaic current flowed through the upper face of the electrode.

4. *Current Delivery.* Current was delivered to the electrodes from a custom-built, computer-controlled power supply (Professional Design and Development Services, Berkeley, CA). Sampling was conducted at 0.25 mA/cm^2; to avoid unnecessary sensation, when the power supply was activated, the current was 'ramped' to its final, constant value over a period of ~30 seconds (a reverse algorithm was used at the end of current passage). To ensure volunteer safety, the voltage drop across the two current delivery electrodes was measured every second and displayed on the computer screen; if the voltage difference exceeded 25 V, the experiment terminated.

5. *Electrolyte.* The background electrolyte, with which the electrode chambers were filled, was 0.1 M NaCl. This provided more than sufficient chloride for efficient operation of the Ag/AgCl electrodes. Although the salt solutions were not buffered, no pH changes were detected in the electrode chambers in any of the experiments performed.

6. *Procedures.* A crucial variable examined in this experiment was the time course of glucose extraction. Of course, with the reverse iontophoresis approach, a "snapshot" of the blood glucose level is not obtained; rather, a cumulative amount of the analyte is extracted over a certain time period, and it is anticipated that this quantity is directly correlated with the average blood glucose level during that same time interval. Reducing the time period of extraction obviously increases the 'resolution' of the approach, but it also diminishes the amount of glucose withdrawn (and places a greater demand upon the analysis of the sample). Current was therefore passed (i.e., extraction was performed) for periods of between 5 and 60 minutes. Periods of longer than 1 hour were not examined because it is unlikely that such an integrated measurement would be of much practical use (blood glucose levels being capable of dramatic changes over such times). Prior to the commencement of an experiment, the skin sites were left in contact with 0.1 M NaCl for 60 seconds. This solution (the "wash") was then removed for analysis and replaced before activating current flow. Several control measurements (no-current) were also made. The most extensive of these involved repeated filling, emptying and replacement of the collection chamber solutions as a function of time. As described below, this allowed determination of the passive extraction of cutaneous glucose.

7. *Analytical.* Glucose assays were performed using a chromatographic system with pulsed amperometric detection [8]. Data were processed as previously described [8].

Solutions were prepared from HPLC water and ACS-grade reagents. To prepare the sample for assay, a 50 μL aliquot was diluted with 150 μL of water; a second 50 μL aliquot was diluted with 150 μL of 0.502 M glucose solution. The two samples were then injected sequentially into the HPLC and the concentration of glucose in the sample was determined by the Method of Standard Addition, which was used to overcome any matrix effects [9]. The detection limit was 0.6 μM (i.e., 1.6 pmol injected).

RESULTS AND DISCUSSION

First, a few comments on the *in vivo* effects of iontophoresis are warranted. Despite the "ramping" protocol, all subjects experienced a mild tingling sensation as the current was brought to 0.25 mA/cm^2. The sensation was typically asymmetric, with more tingling felt at the anode than at the cathode. Generally, the sensation diminished with time of current application, and lasted no longer than 30 minutes. It was found that sensation beneath the larger area electrode chambers was always greater than that below the smaller cells (presumably because of the higher total current passed and the larger number of dermal nociceptors activated). It should be noted, however, that the voltage drop across the electrodes (which was highest at the start of the experiment, but then decreased to a lower, steady value) was similar in the different electrode chamber designs employed; this suggests that, in the 10 cm^2 cells, the current was evenly distributed across the entire area. Iontophoresis, particularly for the longest period of application, caused the skin sites beneath the electrode chambers to become slightly erythematous, and to remain so for 10–60 minutes after termination of current flow. Occasionally, a few, very small, punctate lesions were observable following disappearance of the redness; these marks persisted for several days. These observations are consistent with those that have been reported in the literature [10], and are not considered to be significantly different "side-effects" than those accompanying the use of conventional transdermal drug delivery systems.

Iontophoretic extraction for a period of 60 minutes resulted in data that were apparently self-consistent: extraction at the cathode was most efficient, measurable amounts were also seen at the anode, and the passive and "wash" controls were much smaller. Figure 2 shows the results for three representative subjects and demonstrates the range of responses observed. In total, 9 subjects were studied and the data for the six not shown fall between those of volunteers E and S. The reason underlying the wide variation in extracted glucose quantity is not known at this time. It would appear that the efficiency of reverse electroosmosis is not the same in every individual, presumably reflecting differences in the net skin charge and/or the transport numbers of (e.g.) Na^+ and Cl^- across different individuals' skin. Clearly, in the long term, one is drawn to the conclusion that the potential application of the approach will require a specific calibration for each person.

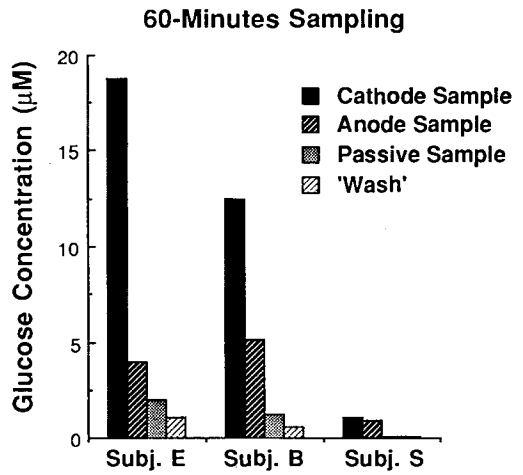

Fig. 2. Iontophoretic sampling, at both anode and cathode chambers, of glucose (compared to passive and "wash" controls) following current passage at 0.25 mA/cm^2 for 60 minutes. Representative data from three subjects are shown.

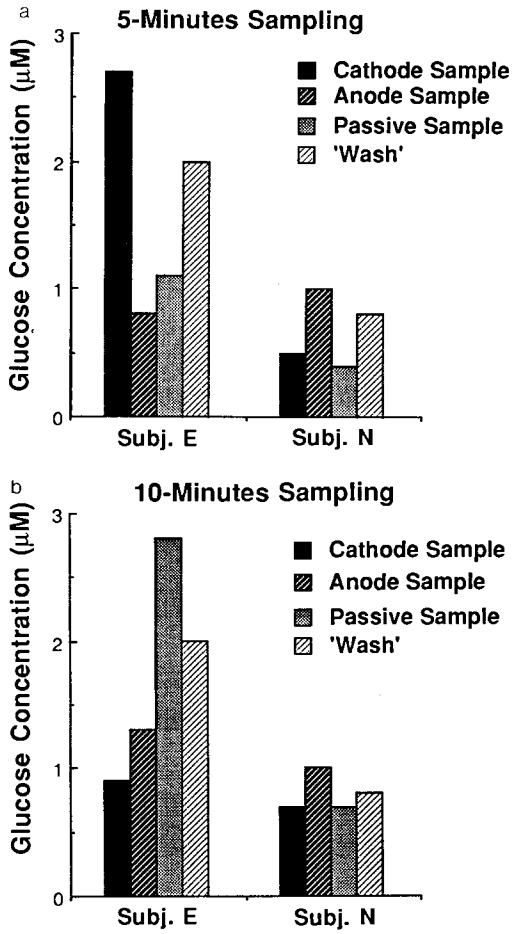

Fig. 3. Iontophoretic sampling, at both anode and cathode chambers, of glucose (compared to passive and "wash" controls) following current passage at 0.25 mA/cm^2 for (a) 5 minutes and (b) 10 minutes. Representative data from two subjects are shown.

When current was passed for shorter periods of time, however, the controls were often as large as (and sometimes greater than) the reverse iontophoretic measurements. Figures 3(a) and 3(b) illustrate representative data following reverse iontophoretic extraction for 5 and 10 minutes, respectively (in this case, 4 or 5 subjects were used at each time point—no clear pattern of response was observed, and the results shown simply exemplify the inconsistency of the

measurements). Clearly, in these cases, it appears that the enhancement of glucose extraction by the electric field, relative to passive diffusion, is negligible.

The detailed passive measurements, as a function of time, were able to shed light on these observations. The rapid availability of glucose in the 'wash' solution, and following only a few minutes of passive extraction, indicated that the stratum corneum (SC), rather than being devoid of glucose under normal circumstances, does in fact contain an appreciable amount of the analyte. A plausible explanation for the presence of glucose in the SC is that it is the product of the deglycosylation of glucosylceramides to ceramides that is known to occur at the stratum granulosum-SC interface [11]. It is unlikely that the level of this glucose is related to the systemic concentration. If the SC does indeed support a steady-state (or quasi-steady-state) concentration of glucose, then the presence of an aqueous "sink" at the skin surface should provoke a solute release process, which will be initially very fast, and then diminish with increasing time. Specifically, the cumulative amount of glucose 'released' should increase linearly with the square-root of time [12]. Figure 4, which shows the results of the 'passive' extraction experiments for 5 individual subjects, indicates exactly the predicted $t^{1/2}$ dependence over a 30-minute period.

This passive release phenomenon became particularly noticeable when the larger area electrode chambers were employed (data from which are presented in Figure 4). Of course, because of the depletion of the glucose 'reservoir' in the SC, the electrotransported flux of glucose ultimately becomes dominant and one observes the expected pattern of results (as illustrated in Figure 2). Quantitatively, we can describe the extraction process as follows:- For the experimental periods studied here (i.e., $t \leq 1$ hour), the cumulative amount of glucose extracted passively (M_p) is given by [12]:

$$Mp = A_s \cdot C_{s,0} \cdot [D_s \cdot t/\pi]^{1/2} \quad (1)$$

where A_s is the area of skin from which glucose is diffusing, $C_{s,0}$ is the analyte's concentration in the SC at $t = 0$, and D_s is its diffusivity. Under the conditions of reverse iontophoresis, on the other hand, glucose will appear at the skin surface according to Eq. (2):

$$M_a = A_i \cdot J_i \cdot t + M_p \quad (2)$$

where M_a is now the cumulative amount of glucose extracted under electrotransport conditions, A_i is the area of the skin through which iontophoretic extraction occurs (and which, presumably, is much less than A_s) and J_i (amount per unit area [of the skin through which iontophoretic extraction occurs] per unit time) is the iontophoretically driven flux at constant current. It follows that, as t increases, and C_s falls, the first term in Eq. (2), which is directly proportional to time (unlike the second which follows $t^{1/2}$) becomes dominant, and the extraction proceeds in a linear, and desirable, fashion. Of course, Eq. (2) is a highly simplified approximation of reality and is presented to illustrate, rather than to analyze precisely, the mechanistic origins of the results obtained. It remains a future goal to develop a rigorous mathematical model with which to interpret more accurately the data.

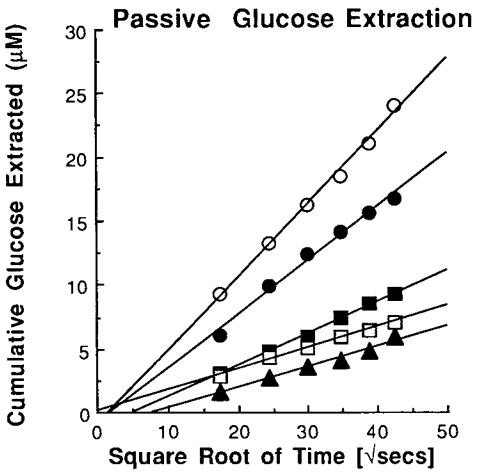

Fig. 4. Cumulative amounts of glucose extracted passively from the skin plotted as a function of the square-root of time. Each set of symbols represents data from an individual subject. The lines drawn through the results are those of linear regression ($r^2 > 0.99$ in all cases).

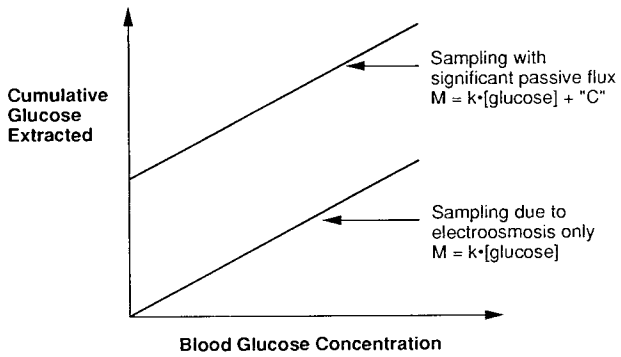

Fig. 5. The calibration issue for noninvasive glucose sampling when there is a significant background signal due to cutaneously-derived analyte.

It follows that the passive data complicate the construction of a calibration plot for the noninvasive extraction of glucose (Figure 5). The longer periods of sampling demonstrate, however, that it should be possible to correlate subdermal glucose levels with extracted amounts. The problem is that the cutaneously-generated glucose provides a background signal that must be overcome by the electroosmotically-drawn material. This requires a finite period of time in the case of an acute measurement. On the other hand, if monitoring is performed continuously (or repeatedly at the same site over the course of several hours, for example), only the initial data will be significantly affected by this background signal.

In conclusion, although the above experiments have highlighted an important constraint in our approach, the successful, *in vivo* demonstration of proof-of-concept is unquestionably encouraging. Optimization of electroosmotic flow and sampling time, improved reproducibility and assay sensitivity, and examination of other potential analytes represent immediate objectives for further investigation.

NOTE ADDED IN PROOF

The reader's attention is brought to subsequent, recently published, material:

(a) J. A. Tamada, N. J. V. Bohannon, R. O. Potts. Measurement of glucose in diabetic subjects using noninvasive transdermal extraction. *Nature Med.* **1**: 1198–1201, 1995.

(b) R. H. Guy. A sweeter life for diabetics? *Nature Med.* **1**: 1132–1133, 1995.

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REFERENCES

1. The Diabetes Control and Complications Trial Research Group. *N. Engl. J. Med.* **329**:977–986, 1993.
2. P. Glikfeld, C. Cullander, R.S. Hinz, and R.H. Guy. U.S. Patent # 5,279,543, "Device for Iontophoretic Non-Invasive Sampling or Delivery of Substances," (1994).
3. R.H. Guy, G. Rao, P. Glikfeld, C. Cullander and R.S. Hinz. U.S. Patent # 5,362,307, "Method for the Iontophoretic Non-Invasive Determination of the In Vivo Concentration Level of and Inorganic or Organic Substance," (1994).
4. R.R. Burnette. Iontophoresis. *In* "Transdermal Drug Delivery: Developmental Issues and Research Initiatives". J. Hadgraft, R.H. Guy, eds. Marcel Dekker, New York, pp. 247–291, 1989.
5. M.J. Pikal. The role of electroosmotic flow in transdermal iontophoresis. *Adv. Drug Del. Rev.* **9**:201–237, 1992.
6. G. Rao, P. Glikfeld, R.H. Guy. Reverse iontophoresis: Development of a noninvasive approach for glucose monitoring. *Pharm. Res.* **10**: 1751–1755, 1993.
7. P. Green, R. S. Hinz, C. Cullander, G. Yamane and R. H. Guy. Iontophoretic delivery of amino acids and amino acid derivatives across the skin in vitro. *Pharm. Res.* **8**: 1113–1120, 1991.
8. W.R. LaCourse. Pulsed electrochemical detection in high performance liquid chromatography. *Analysis* **21**: 181–195, 1993.
9. J.C. Miller and J.N. Miller. *Statistics for Analytical Chemistry*. John Wiley & Sons, New York, 1986, pp. 100–102.
10. P.W. Ledger. Skin biological issues in electrically enhanced transdermal delivery. *Adv. Drug Delivery Rev.* **9**, 289–307, 1992.
11. N.Y. Schurer, P.M. Elias. The biochemistry and function of stratum corneum lipids. *Adv. Lipid Res.* **24**: 27–56, 1991.
12. J.C. Crank. *The Mathematics of Diffusion* (2nd Edition). Oxford University Press, Oxford, 1976.