**Lithium Monitoring by Reverse Iontophoresis in Vivo**

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**Background:** We investigated reverse transdermal iontophoresis as an alternative, noninvasive method for lithium monitoring in vivo. The objectives of such an approach would be to facilitate compliance with treatment and to improve the quality of life for bipolar patients.

**Methods:** We studied 23 bipolar or schizo-affective patients. Over a 2-h period, we extracted lithium and other cations across intact skin by application of an electric current (0.8 mA) and quantified the concentrations by ion chromatography. A blood sample provided comparative reference values for the drug and other electrolytes.

**Results:** Lithium, sodium, potassium, and calcium were efficiently extracted by iontophoresis. Lithium extraction fluxes were proportional to the corresponding serum concentrations, whereas sodium, potassium, and calcium extraction fluxes were relatively constant, consistent with their stable concentrations in blood. Normalization of the lithium extraction flux with that of sodium, which acted as an “internal standard”, permitted calibration of the monitoring procedure without the need for a blood measurement. This conclusion was tested retrospectively by dividing the patients into two groups. The reverse iontophoretic extraction data from the first subset (a) established the proportionality between lithium iontophoresis (or the relative electrophoretic transport of lithium and sodium) and (b) predicted lithium blood concentrations in the second subset of patients. The predictive ability was very good, with the internal standard concept providing substantial benefit.

**Conclusions:** Reverse iontophoresis appears to offer a novel and accurate method for lithium monitoring.

Fifty years after the first report of its antimanic effects (1), lithium remains a first-choice mood stabilizer for preventing relapses in bipolar disorders (1–3). It is also the only mood stabilizer for which a preventive effect on suicidal risk has been clearly shown (3, 4). Regular lithium monitoring is essential to ensure efficacy and to prevent adverse effects (5). Although steady-state plasma concentrations of the drug are achieved in a few days, a therapeutic response, e.g., in the treatment of manic episodes, is not observed until 2–3 weeks after initiation of treatment (6). Serum concentrations are therefore monitored on a weekly basis at the beginning of treatment, then monthly or at even longer intervals (7). More frequent supervision is required (a) in the absence of a satisfactory therapeutic response, (b) in the presence of adverse effects or diseases that affect drug disposition, (c) to verify compliance (~40% poor compliance has been reported for bipolar patients) (8), or (d) when the polypharmacotherapy involves potentially interacting drugs (9). Lithium monitoring involves a blood test that provides the so-called “Standardized 12 h Li” Serum Concentration”: i.e., patients are subjected to a blood draw in the early morning, ~12 h after the last dose of the previous day and before the first administration on the day of monitoring.

It can be argued, therefore, that a completely noninvasive technique for lithium monitoring would facilitate compliance with treatment and improve the quality of life for bipolar patients. Saliva has been considered as an alternative matrix for lithium monitoring, but the broad inter- and intraindividual variability of the saliva/plasma concentration ratio severely limits the usefulness of this approach (10, 11). The skin offers, in principle, an extensive and accessible surface for drug sampling, and early research demonstrated the transdermal efflux of substances into collection patches by passive diffusion and secretion in the sweat (12–14). However, because these
mechanisms are slow and inefficient, the long accumulation time required before the drug can be detected in the patch renders the method impractical for pharmacokinetic tracking (15).

Transdermal flux can be dramatically increased, however, by iontophoresis, in which a low electrical current (<0.5 mA/cm²) is applied to the skin to facilitate transport of polar and charged molecules through the skin barrier (16). Initially developed for transdermal drug delivery, iontophoresis has also been investigated as an alternative, noninvasive sampling technique. Indeed, The US Food and Drug Administration recently approved a “reverse” iontophoretic device (Glucowatch® Biographer®; Cygnus Inc.) that monitors glycemia for 12 h to help in the management of diabetes (17). The potential application of this technique for the monitoring of drugs and clinical markers has been reviewed recently (18). Two mechanisms of transport are involved in iontophoresis. Electromigration involves the movement of ions, which carry charge across the skin and are driven (attracted) specifically toward the electrode of opposite polarity. Electroosmosis is a net solvent flow in the anode-to-cathode direction, which enables much improved permeation of neutral species (e.g., glucose) and further enhances cationic transport (19).

Lithium monitoring by reverse iontophoresis has been investigated in vitro (20). This small, mobile, cationic drug is an excellent candidate for iontophoretic extraction. Lithium is transported by electromigration to the cathode (negative electrode) at a flux (J Li, nmol/h) described by Eq. 1:

\[ J_{Li} = \frac{I \times t_{Li}}{F \times z_{Li}} \]  

(1)

Where \( t_{Li} \) and \( z_{Li} \) are the transport number and valence (+1) of the drug, respectively; \( F \) is Faraday’s constant; and \( I \) is the current applied. The transport number of a given ion (i.e., the fraction of charge transported through the skin by that ion) is proportional to its concentration when competing co-ions are present (21). This is obviously the case in reverse iontophoresis, in which the physiologic milieu provides a panoply of competing co-ions. Under these circumstances, Eq. 1 can be alternatively expressed as:

\[ J_{Li} = \gamma \times c_{Li} \]  

(2)

where \( c_{Li} \) is the subdermal concentration of lithium, and \( \gamma \) is a constant. The in vitro investigation demonstrated a linear relationship between \( J_{Li} \) and \( c_{Li} \) (20). In vivo, on the other hand, it is important to correlate \( J_{Li} \) with the serum concentration of the drug. Although it is perhaps reasonable to suppose that the subdermal compartment is in rapid (or even instantaneous) equilibrium with the blood for a hydrophilic compound, such as lithium, it was nevertheless an important initial objective of this study to verify this hypothesis. To this end, only individuals undergoing chronic lithium therapy were admitted into the study, ensuring that any impact of slow drug distribution kinetics would be minimized.

Iontophoresis is not a selective extraction process; in fact, all ions present in the system compete for transporting the charge (22). Only those ions with a sufficiently high transport number will be efficiently extracted. The competitive nature of the extraction process and the complex composition of the in vivo milieu mean that transport numbers cannot be predicted a priori. If the efficiency of lithium extraction (characterized by the constant \( \gamma \) in Eq. 2) shows wide inter- and inrasubject variability, calibration by use of the concentration in blood is obligatory. Such is currently the case for the Glucowatch Biographer, for example. In the case of therapeutic lithium monitoring, reverse iontophoresis offers no advantage if calibration in this way is necessary. Additional objectives of the present study, therefore, were on the one hand to examine the degree to which \( \gamma \) varies among individuals and, on the other, to explore an “internal standard” concept (20, 23, 24) as a noninvasive means with which to standardize the reverse iontophoresis of lithium.

An internal standard (IS) is defined as an endogenous compound that is present at relatively constant systemic concentration. It follows that the ratio of extraction fluxes \( J_{Li}/J_{IS} \) should be directly proportional to the ratio of subdermal concentrations, \( c_{Li}/c_{IS} \). In other words, and given that \( c_{IS} \) is constant:

\[ J_{Li}/J_{IS} = \gamma \times c_{Li} \]  

(3)

Thus, the simultaneous reverse iontophoretic extraction of Li and the internal standard enables the concentration of the analyte of interest to be directly determined once \( \gamma \) is known. The validity of this hypothesis has been demonstrated in vitro for lithium, with sodium and potassium used as internal standards (20). We tested the internal standard calibration in vivo and evaluated the suitability of the major cationic electrolytes, i.e., sodium, potassium, calcium, magnesium, and ammonium, as internal standards.

**Materials and Methods**

**MATERIALS**

L-Histidine (USP grade, Eur.Ph. grade), sodium chloride (Eur.Ph. grade), silver wire (99.9% purity), silver chloride (99% purity), and platinum (99.9% purity) were purchased from Sigma-Aldrich. Deionized water (resistivity \( \geq 18.2 \, \text{MΩ/cm} \) was used to prepare all solutions.

**PATIENTS**

Thirty ambulatory patients diagnosed with bipolar or schizo-affective disorder entered the study. The patients were receiving chronic lithium therapy at doses of 12–36 mmol/day (sulfate or carbonate salt), for no fewer than 3 weeks. Their last dose of lithium was taken the day before the iontophoresis experiment between 1900 and 2100 in the evening. Of the original patients enrolled, data from
six were unusable (because of current interruption during iontophoresis, a leak from the collection chamber, or a missing blood sample). The results presented below, therefore, correspond to 24 patients (11 women and 13 men; age range, 20–59 years).

EXPERIMENTAL PROTOCOL
The clinical protocol was accepted by the internal review board of the Geneva University Cantonal Hospital. Informed consent was obtained from all participants. Patients arrived at 0800 and were maintained in supine position throughout the study, which took place from 0800 to 1030. The arm presenting better venous accessibility was reserved for the standard monitoring procedure. The other forearm was used for reverse iontophoresis. A glass cylinder, which served as the cathodal compartment, was fixed to the forearm (at a site that had been cleaned with an alcohol swab) by use of a Teflon ring. Silicone grease was applied to avoid leaks. The apparatus was held firmly in place with medical tape (3M Healthcare). The area of transport through the skin was 3.2 ± 0.4 cm². The glass cell was filled with 1.2 mL of an aqueous solution containing 10 mmol/L histidine (pH 7.47) for 1 min; this liquid was then completely removed and refreshed, and a Ag/AgCl electrode was then inserted into the solution and maintained at least 5 mm from the skin surface by use of a plastic top that covered the cell.

The anodal chamber was either a similar glass cell, again equipped with a Ag/AgCl electrode and filled with 62 mmol/L NaCl solution, or a 32-cm² commercially available adhesive iontophoretic patch (Dispersive Pad, Iogel Medium; Iomed). The anodal electrode was applied on the same forearm at ∼10 cm from the glass cell holding the cathode.

A Phoresor II Auto (PM850; Iomed) was used to deliver a constant direct current of 0.8 mA. Iontophoresis was performed for four 30-min intervals. At the end of each interval, the current was stopped, and the electrode solutions were entirely removed and refreshed. The electrode solutions were analyzed for lithium and other cations as described below.

The Standardized 12 h Li⁺ Serum Concentration blood sample was taken 90–100 min after initiation of iontophoresis and was reserved for subsequent analysis (see below).

ANALYTICAL METHODS
Ionic chromatography was used to quantify lithium, sodium, potassium, calcium, and magnesium in the electrode chamber solutions. A Dionex DX-600 system (Dionex) equipped with a GP-50 pump, a AS-50 thermostated compartment (40 °C), an ED-50 detector, a CS-16 cationic column, and an Atlas suppressor was used. Data analysis was performed with Chromeleon, Ver. 6.4, software (Dionex). Lithium, sodium, and potassium were separated with a mobile phase containing 6.25 mmol/L methanesulfonic acid at a flow rate of 2 mL/min and a suppressor current of 42 mA. Calcium and magnesium were separated with a mobile phase containing 25 mmol/L methanesulfonic acid at a flow rate of 1 mL/min and a suppressor current of 83 mA. Calibration curves were constructed before each set of analyses. The quantification limit (10 times the background noise) was 1 μmol/L for lithium, and the imprecision (CV) was 0.1% and 2.5%, respectively, for the smallest and highest concentrations used to construct the calibration curve (6.25–100 μmol/L; n = 6).

Lithium was quantified in the blood samples with an ion-selective electrode (Cobas Integra Model 700; Roche Diagnostics). Sodium, potassium, and calcium were also quantified by use of ion-selective electrodes (Synchro LX20; Beckman Coulter Inc.). Magnesium and ammonium were measured (Synchro LX20) by colorimetric and enzymatic tests, respectively.

DATA ANALYSIS AND STATISTICS
Transport numbers, or fraction of charge transported by each ion, were calculated for each sampling interval as follows:

\[ t_i = \frac{m_i \times z_i}{I \times t / F} \]  

Where \( t_i \) and \( z_i \) are the transport number and valence of the ion ; \( m_i \) is the number of moles of the ion \( i \) extracted in an interval of time \( t \) (seconds); \( I \) is the intensity of electrical current applied in amperes (Coulombs/s); and \( F \) is Faraday’s constant (96500 Coulombs/mol).

All data are shown as the mean (SD) unless otherwise indicated. Data analysis and linear regression were performed with Graph Pad Prism, Ver. 3.02 (GraphPad Software). Repeated-measures ANOVA was used to compare ion fluxes at different times. The level for statistical significance was set at \( P < 0.05 \). All regressions presented in this work were significant \( (P < 0.0001) \) unless otherwise stated. Confidence intervals were calculated as described elsewhere (25).

Results and Discussion
The serum concentrations of lithium, sodium, potassium, magnesium, calcium, and ammonium are presented in Fig. 1. Lithium concentrations were in the range typical of patients being treated at the Geneva University Hospital, with ∼75% having steady-state concentrations between 0.5 and 0.9 mmol/L. The rest had lower concentrations, considered to be out of the therapeutic range, presumably because of poor compliance. Almost all serum concentrations of the other cations were inside the normal physiologic ranges. The Kolmogorov–Smirnov normality test demonstrated that the distributions of the concentrations of all ion concentrations considered were normal (\( P < 0.05 \)). The CVs were 1.7% for sodium, 6.1% for potassium, 6.2% for magnesium, and 3.6% for calcium. The
corresponding value for ammonium was >65%; consequently, we focused no further attention on this species.

The extraction procedure began with a 1-min "washing" period during which current was not applied. This washing step serves as an indicator of the existence (or not) of ion reservoirs in the more superficial layers of the skin (a point discussed further below). All washing samples contained detectable amounts of sodium and potassium, whereas lithium and calcium were detected in 22 and 16, respectively, of the 24 samples analyzed. Magnesium was rarely detected (only two samples). The amounts recovered were 14.4 (14.2) nmol of lithium, 163 (212) nmol of sodium, 106 (140) nmol of potassium, and 10.5 (10.2) nmol of calcium.

Iontophoresis was well-tolerated by the patients. Pricking sensations were frequently reported, being more noticeable at the anode at the start of each current interval. A mild, uniform, transient redness (typically resolved within a few hours) was observed at both electrode sites at the end of the protocol, similar to that observed in previous reports (26, 27).

IONIC FLUXES AND TRANSPORT NUMBERS

Lithium was efficiently recovered at the cathode for all patients. In the first six patients, the anode chamber was also analyzed for lithium, and although a measurable amount was found, the calculated flux never exceeded 6% of the amount that migrated to the cathode. Subsequently, for practical reasons, disposable electrode patches were used for the anode in the remaining patients. In preliminary control experiments, the technique was also tested on untreated individuals. Iontophoretic extraction periods of 0.5, 1, and 2 h were examined and produced no quantifiable amounts of lithium. If current passage was continued for 3 h (i.e., six times the duration of each extraction period used in this study), the apparent endogenous flux of lithium was barely 1% of that seen, on average, in the patients.

The reverse iontophoretic extraction fluxes measured for each cation in the four successive 30-min periods of current passage are shown in Fig. 2. The mean (SD) lithium flux (Fig. 2A) was 90.5 (37.9) nmol/h for the first sampling interval and was significantly higher (P < 0.001) than those measured subsequently [56.7 (18.6) nmol/h]. This higher initial extraction, plus the fact that lithium was present in the pretreatment washing solution, suggests the existence of a lithium reservoir in the skin and/or skin appendages. Lithium has been found in cutaneous lesions (rashes and ulcers) and in biopsies obtained from the epidermis, dermis, and subcutaneous adipose tissue of patients and guinea pigs chronically treated with the drug (28, 29). It has also been demonstrated that lithium is secreted in sweat (30, 31); thus, lithium found in the washing samples could also originate from this source. The apparent accumulation of lithium in the skin may lead to the creation of a reservoir, the magnitude of which is unrelated to the serum concentration of the drug. Because the epidermis is avascular, it obtains all of its nutrients from the dermal microcirculation by diffusion, and the mechanism by which lithium arrives in the epidermal layers is most likely similar. It is possible, therefore, that as keratinocytes undergo differentiation and ultimately form the stratum corneum (the outermost layer of the skin) a small fraction of the lithium circulating systemically becomes “trapped” in this upper skin compartment. The amount accumulated would vary among patients as a function of skin perfusion and epidermal turnover. Depletion of this reservoir would explain the early higher fluxes, and the fact that the extraction then stabilizes at a constant lower concentration. In vitro, this phenomenon has not been observed (20); in that study, the skin used was obtained from pigs that had never been exposed to the drug. Under these circumstances, the extraction flux, not unexpectedly, increases toward its steady-state value. Parenthetically, it is worth noting that a skin reservoir has also been identified for glucose (in this case, it is believed, attributable to metabolic breakdown of skin lipids) and that it is therefore necessary to deplete this material before calibration and operation of the Glucowatch (27).

Sodium extraction showed a different pattern (Fig. 2B). The flux in the first interval [12.9 (1.6) µmol/h] was significantly lower (P < 0.001) than the subsequently achieved stable value [15.8 (1.3) µmol/h]. Potassium

![Fig. 1. Box-and-whisker representation of the measured serum concentrations of lithium, sodium, potassium, calcium, and magnesium for 24 patients. Dotted lines indicate the "normal" range observed (35). Note that the range for lithium is that currently adopted by the Department of Psychiatry of the Geneva University Hospital.](image-url)
transport slightly but significantly \( (P \leq 0.001) \) decreased from 4.4 (1.2) to 3.8 (0.8) \( \text{mol/h} \); the flux of this cation was more variable than that of sodium: the CVs were 21% and 8%, respectively, for the last three sampling periods. Calcium extraction did not evolve in any clear pattern, the flux decreasing from 172 (37) \( \text{nmol/h} \) at 30 min to 156 (24) at 60 min and finally reaching 185 (37) \( \text{nmol/h} \) at 2 h. The CV associated with calcium transport was also high (18%). Finally, magnesium extraction fluxes, although detectable, were below the limit of quantification. The lower fluxes measured for the divalent calcium and magnesium ions are consistent with Eq. 1.

The a priori prediction of transport numbers remains challenging, particularly in reverse iontophoresis where the spectrum of potential charge carriers from within the body to the skin surface is very broad. In general terms, however, it is known that the transport number of a specific ion will depend on its concentration and mobility relative to the concentrations and mobilities of the competing ions in the system \((21, 22)\). For this reason, sodium is by far the principal charge carrier, being a mobile cation present at a much higher concentration than all others. Its transport number \([t_{Na} = 0.54 (0.04)]\) and that of lithium \([t_{Li} = 0.0018 (0.0006)]\) were quite consistent with the corresponding values determined in vitro \((20)\) in an experiment designed to mimic, to some extent, the in vivo situation examined in this work. The transport number of potassium \([t_{K} = 0.13 (0.03)]\) was higher than that measured in vitro \([0.042 (0.006)]\) \((20)\) but was consistent with other values previously determined in vivo \((32)\).

**RELATIONSHIP BETWEEN SERUM LITHIUM CONCENTRATIONS AND IONTOPHORETIC EXTRACTION FLUXES**

A key practical objective of this study was to establish whether a clear relationship existed between systemic lithium concentrations and reverse iontophoretic extraction rates across the skin. Fig. 3 illustrates how the serum lithium concentrations, measured \( \approx 90 \text{ min after the initiation of current passage, compared with the iontophoretic fluxes of the drug for the four different, 30-min periods of extraction. Linear regression of these data gave the correlations in Table 1. Although it is clear that there is a very poor relationship between the serum concentrations and the extraction data from the first sampling period, the correlations thereafter are excellent.**
on the systemic concentration of the drug. Although it is certainly logical to expect that the iontophoretic flux should correlate better at times closer to the blood sampling, the terminal half-life of lithium \( t_{1/2} \) (5) is sufficiently long that the concentration 30 min after initiation of reverse iontophoresis would only be slightly different from that 1 h later and unquestionably not so divergent as to explain the poor correlation with the first period of iontophoresis relative to the later measurements.

Nevertheless, the results shown in Fig. 3 and Table 1 are overwhelmingly positive in terms of establishing the in vivo relationship proposed between lithium concentrations and reverse iontophoretic extraction flux. After 1 h of current passage, excellent correlations were obtained, with data from both patients with “normal” drug concentrations, within the therapeutic window, and from those with low lithium concentrations. The challenge for a practical monitoring system will clearly include minimizing the time necessary to deplete the reservoir of lithium in the skin and shortening as far as possible the time of current passage necessary for reliable and sensitive analysis of the drug in the collection device.

Panels C and D in Fig. 3 highlight statistically aberrant

**Table 1.** Linear regressions, at different times of iontophoresis, of the extracted lithium flux \( J_{Li} \); data in Fig. 3), and the ratio of extracted lithium and sodium fluxes \( J_{Li}/J_{Na} \), as a function of the serum drug concentrations, according to Eqs. 2 and 3, respectively.\(^a\)

<table>
<thead>
<tr>
<th>Time, min</th>
<th>( \gamma ) (SE), ( \mu L/h )</th>
<th>Intercept, nmol/h</th>
<th>( r^2 )</th>
<th>( \gamma_{Na} ) (SE), L/mol</th>
<th>Intercept</th>
<th>( r^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>136 (38)</td>
<td>11.5</td>
<td>0.38</td>
<td>11.7 (3.0)</td>
<td>4 \times 10^{-4}</td>
<td>0.42</td>
</tr>
<tr>
<td>60</td>
<td>107 (11)</td>
<td>−0.3</td>
<td>0.82</td>
<td>6.9 (0.4)</td>
<td>5 \times 10^{-5}</td>
<td>0.93</td>
</tr>
<tr>
<td>90</td>
<td>100 (7)</td>
<td>−4.0</td>
<td>0.91</td>
<td>6.1 (0.4)</td>
<td>−2 \times 10^{-4}</td>
<td>0.92</td>
</tr>
<tr>
<td>120</td>
<td>92 (7)</td>
<td>−0.2</td>
<td>0.90</td>
<td>5.6 (0.3)</td>
<td>7 \times 10^{-5}</td>
<td>0.93</td>
</tr>
</tbody>
</table>

\(^a\) Results are based on data from 23 patients.
data (Grubbs’s test, \(P = 0.01\)), which were not used in the overall statistical analyses presented here, from two patients (for this reason, the number of individuals indicated in the results is sometimes slightly different). The reverse iontophoretic extraction flux from one of these patients was exceptionally high during the two later periods of iontophoresis; for the other volunteer, only the result in the final period was abnormally large. Precise explanations for these observations are not available. Perhaps the former patient had an exceptionally large skin reservoir of the drug; possibly the outlier for the second patient was the result of an accidental contamination. Clearly, additional studies, in much larger populations, will ultimately be necessary to provide information about intra- and intersubject variability and potential sources of systematic and opportunistic error.

The linear correlation between lithium extraction fluxes and the corresponding serum concentrations provides a quantitative measure of the proportionality constant (\(\gamma\)) linking the two variables (Eq. 2). The most direct evidence of the potential of reverse iontophoresis for lithium monitoring would be to demonstrate that \(\gamma\) varies very little both between different individuals and within each person tested. That is, it would be possible then to derive a “global” value of \(\gamma\) that could be used in all patients, with high confidence, to convert the reverse iontophoretic extraction flux to a serum concentration. The correlation based on the first 30-min period of extraction would obviously be unacceptable (see Fig. 4 and Table 1): \(\gamma\) had a CV of 28%. However, for the next extraction period, the CV was 10%, and in the two final intervals, it decreased further to \(\sim 7\%\). Compared with glucose extraction by use of electroosmotic flow induced by iontophoresis, this variability in the proportionality constant is extremely low and reflects the relative stability of the transport numbers of the competing cations as described earlier. In other words, the fact that the concentrations and mobilities of these ions are reasonably constant in vivo (or at least within sufficiently limited ranges such that their electrotransport kinetics are not too variable) means that the lithium extraction rates will respond to differences in the serum concentration in a rather consistent fashion from patient to patient. If confirmed in a much larger group of patients, the variability in \(\gamma\) may, in theory, be small enough to provide a general “calibration factor” for the direct therapeutic monitoring of lithium by reverse iontophoresis.

**INTERNAL STANDARD CALIBRATION**

Recognizing, however, that the straightforward approach proposed above carries with it an obvious risk of data verification, we examined the internal standard calibration idea. The value of this strategy had been demonstrated previously in vitro, with sodium and potassium cations used as the potential internal standards. Physicochemically much like lithium, these ions are small and mobile and are transported across the skin primarily by electromigration.

A key criterion for any one of these cations to act as an internal standard for lithium is that its iontophoretic extraction flux be constant. For sodium, this was indeed the case: its transport number, as the principal charge carrier, was high (\(\sim 0.54\)), with a CV of \(\sim 8\%\). In contrast, the transport numbers for potassium and calcium were much more variable, with CVs of 15–25%. Again, it is not entirely clear why we observed such wide variability. From a purely mechanistic standpoint, given the relatively constant systemic concentrations of these (albeit) secondary charge carriers, one would have expected more consistent behavior (as was in fact observed for lithium). A possible explanation lies in the fact that concentration gradients for calcium and potassium exist naturally in the epidermis. According to the literature (33, 34), calcium concentrations increase significantly from the basal epidermis to the outer stratum granulosum and then decrease precipitously in the stratum corneum, whereas potassium also increases up to a maximum just below the stratum granulosum before falling off dramatically across the most superficial layers of the barrier. It is possible, therefore, that iontophoresis disturbs these ion concentration gradients (and presumably the intracellular/extracellular distribution of these ions as well) and provokes a subsequent attempt by the skin to reestablish the “normal” situation. Consequently, it is conceivable that a competitive and dynamic situation is induced as iontophoresis extracts these ions to the surface and the skin strives to maintain the status quo. Resolution of this point requires considerable further study.

When we tested the applicability of Eq. 3, using the measured systemic concentrations of sodium, potassium, and calcium and their respective reverse iontophoretic flux ratios with lithium, only the lithium/sodium ratios gave acceptable results. Table 1 shows the linear regression results and clearly demonstrates that, after 30 min of iontophoresis, the ratio \(J_{Li}/J_{Na}\) should be usefully predic-
tive of the serum lithium concentration ($r^2 > 0.92$). As observed for $\gamma$ (the proportionality constant linking $J_{Li}$ to $c_{Li}$), $\gamma^*$, which links $J_{Li}/J_{Na}$ to $c_{Li}$, becomes very stable after the first iontophoresis period, with a low error. In contrast, for potassium and calcium, the correlations between $J_{Li}/J_K$ and $c_{Li}$ and between $J_{Li}/J_{Ca}$ and $c_{Li}$ were much less impressive: in the former case, the $r^2$ values at 90 and 120 min were 0.68 and 0.58, respectively; in the latter, the corresponding values were 0.80 and 0.64. It can be reasonably concluded, therefore, that sodium is by far the most suitable internal standard for “calibration-less” lithium monitoring.

### Prediction of Serum Lithium Concentration

In a final component of the investigation, we examined the potential ability of the relationships derived to predict serum lithium concentrations from reverse iontophoretic extraction flux data. Retrospectively, the patients were divided into two groups: The first 10 patients acted as a “training set”, the data from which were used to develop population values of $\gamma$ and $\gamma^*$ (specifically, from the regressions of $J_{Li}$ and $J_{Li}/J_{Na}$, respectively, against $c_{Li}$). The results of this exercise are summarized in Table 2A. With these proportionality constants and the measured values of $J_{Li}$ and $J_{Li}/J_{Na}$ in the remaining patients (the “test group”), the $c_{Li}$ values were predicted and then compared with the actual, experimentally determined results. The outcome of this exercise is presented in Table 2B, which contains the results of simple linear regressions between the predicted and measured values of $c_{Li}$ using the data from the two later periods of iontophoretic extraction. The correlations are excellent, and the slopes closely approach the theoretical values of unity (particularly for final interval of iontophoresis).

The approach was then better characterized by an inverse prediction procedure (25), which allowed the 95% confidence interval associated with each predicted value to be determined. Fig. 5 shows a comparison of the experimentally measured $c_{Li}$ with the predicted results (together with the corresponding 95% confidence interval) based on the training set values of $\gamma$ and $\gamma^*$. In the former case, the average confidence intervals at 1.5 and 2 h of iontophoresis were ±0.14 and ±0.19 mmol/L, respectively. When $\gamma^*$ was used, the corresponding values decreased to ±0.08 and ±0.11 mmol/L. It is apparent that the predictability is rather good, with the iontophoretic extraction data at 1.5 h providing systematically narrower confidence intervals independent of whether $\gamma$ or $\gamma^*$ was used for the prediction. It is also important to point out that normalization of $J_{Li}$ with the sodium extraction flux almost halved the average confidence interval associated with the prediction of $c_{Li}$, lending credibility and value to the internal standard calibration approach.

However, close inspection of Fig. 5 shows that the confidence intervals for patients 18, 19, and 21 are large. These patients had the lowest measured serum concentrations of lithium (0.16–0.32 mmol/L), whereas the training set data were in the range 0.42–0.79 mmol/L. Statistically speaking, therefore, these three patients were outside the predictive capabilities of the model (25), and this emphasizes the importance of considerably expanding the database to ensure that the training set encompasses the widest possible range of $c_{Li}$. Nevertheless, it can be argued that this exercise has been generally successful and positive and that a clear value for the internal standard, in terms of improving data and in terms of predictive quality, has been demonstrated.

In conclusion, this work shows that lithium can be easily extracted by transdermal iontophoresis in a concentration-dependent manner. The variability of the extraction process is relatively low and allowed (admittedly limited)

### Table 2. Linear regressions for the training set (A) and between the predicted and measured values of $c_{Li}$ in the test group (B).

<table>
<thead>
<tr>
<th>A. Training set.</th>
<th>$J_{Li}$ vs $c_{Li}$</th>
<th>$J_{Li}/J_{Na}$ vs $c_{Li}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time, min</strong></td>
<td>$\gamma$ (SE), $\mu$L/h</td>
<td>Intercept, mmol/h</td>
</tr>
<tr>
<td>30</td>
<td>181 (69)</td>
<td>−11</td>
</tr>
<tr>
<td>60</td>
<td>112 (22)</td>
<td>0</td>
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<tr>
<td>90</td>
<td>114 (15)</td>
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<td>120</td>
<td>90 (17)</td>
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<tr>
<th>B. Test group.</th>
<th>Prediction from $J_{Li}$ and $\gamma$</th>
<th>Prediction from $J_{Li}/J_{Na}$ and $\gamma^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time (min)</strong></td>
<td>Slope (SE)</td>
<td>Intercept, $\mu$L/L</td>
</tr>
<tr>
<td>90</td>
<td>0.83 (0.07)</td>
<td>93</td>
</tr>
<tr>
<td>120</td>
<td>0.96 (0.09)</td>
<td>−31</td>
</tr>
</tbody>
</table>

*The linear regressions in Table 2A, for different times of iontophoresis, are calculated based on the extracted lithium flux ($J_{Li}$; data from Fig. 3) and the ratio of extracted lithium and sodium fluxes ($J_{Li}/J_{Na}$) as a function of $c_{Li}$, according to Eqs. 2 and 3, respectively. Results are based on data from patients 1–10, the training set. Linear regressions in Table 2B are between the predicted and measured values of $c_{Li}$ in patients 11–23 (the test group) calculated with either $J_{Li}$ or $J_{Li}/J_{Na}$ and the values of $\gamma$ and $\gamma^*$ derived from the training set for the two later periods of iontophoretic extraction.*
predictive population extraction parameters to be estimated. The use of sodium as an internal standard decreases the error associated with the predicted serum values. The results support the premise, therefore, that transdermal iontophoresis constitutes an alternative to blood sampling for lithium monitoring in vivo. From a practical standpoint, considerable optimization of the approach is necessary, particularly with respect to maximizing extraction fluxes to shorten measurement times and in terms of facilitating sample collection and analysis.

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References

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