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Determination of Trace Lithium in Human Erythrocytes by Electrothermal Atomic-absorption Spectrometry with Pyrocoated Graphite Tubes and Integrated Platform

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Abstract

Electrothermal graphite-furnace atomic-absorption spectroscopy with pyrocoated graphite tubes, integrated platform and matrix modification was used to determine submicromolar concentrations of trace lithium in human red blood cells.

Matrix-matched samples were used to establish calibration curves for concentrations up to 0·58 μM (addition-calibration method) with satisfactory linearity ($r^2 > 0.99$) and intra- and inter-day variability (CV<11·4%). The median concentration of trace lithium in the cells of 40 healthy Caucasian volunteers devoid of medical or psychiatric history was 0·23 μM (inter-quartile range 0·20–0·30). The levels of trace lithium in the red blood cells correlated ($r^2 = 0.83$) with plasma concentrations (median 0·13 μM, inter-quartile range 0·11–0·19) measured in the same blood sample. Dietary factors (e.g. consumption of lithium-containing mineral water) affected both levels. The red blood cell/plasma lithium ratio had a median value of 1·57 (inter-quartile range 1·16–2·07), implying that trace lithium is accumulated in erythrocytes. This contrasts with most reports of red blood cell/plasma ratio, measured during therapeutic treatment with lithium, for which the average value is 0·5–0·8, albeit for much higher concentrations of lithium (approx. 500–800 μM).

The proposed analytical method has the required sensitivity and accuracy for determination of trace lithium in red blood cells and makes it possible to perform epidemiological studies to assess human exposure to environmental lithium in diet and beverages, and inter-individual variations in trans-membrane and renal lithium kinetics at the submicromolar level.

Although lithium is an effective drug for the treatment of mania, its mechanism of action is still unclear (Price & Heninger 1994) and the reasons for the inter-individual variability of response to lithium therapy remain controversial (Greil et al 1977). On the basis of in-vitro and in-vivo studies performed with red blood cells exposed to pharmacological or therapeutic concentrations of lithium, variations of the Na^+-dependent Li^+ counter-transport system across membranes have been incriminated (Motas de Freitas et al 1991). It was therefore hypothesized that Li^+ concentrations in red blood cells correlate better with brain lithium levels than do extracellular (i.e. plasma) levels. Moreover, the steady-state ratio of red blood cell/plasma lithium during prophylactic lithium therapy has been reported by some authors (Ostrow et al 1978; Ramsey et al 1979) to have prognostic value for therapeutic response, although this has not been confirmed by others (Richelson et al 1986). Interestingly, abnormalities of Li^+ transport across red blood cells have also been reported in hypertensive patients (Canessa et al 1980).

Lithium is present at trace levels ($\mu g\,L^{-1}$ levels, approximately 5000 times lower than therapeutic levels) in the biological fluids of untreated individuals. In renal patho-physiological studies trace lithium was shown to be a valuable marker of the extent to which sodium and water are re-absorbed at the proximal tubule level (Steinhäuslin et al 1994; Magnin et al 1996 and literature cited therein). Although a low-lithium diet adversely affects the growth of animals (Anke et al 1991) it is

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unclear whether it is an essential element for man. Environmental and dietary lithium have been claimed to modulate the incidence of psychiatric diseases, suicide and crime in populations (Dawson 1991).

In contrast, neither trace lithium levels on plasma nor trace lithium erythrocyte/plasma ratios for untreated patients suffering from affective disorders have been directly assessed or compared with those of healthy subjects. This prompted us to undertake a study of possible alterations of intra- and extracellular endogenous lithium kinetics in psychiatric patients compared with controls. A sensitive analytical method was required for the determination of physiological levels of lithium in plasma and erythrocytes.

Graphite-furnace atomic-absorption spectrometry, with standard L'Vov pyrolytically-coated graphite tubes in combination with ammonium nitrate matrix modification, has already been used in our laboratory for quantitative determination of endogenous submicromolar concentrations of lithium in human plasma and urine (Magnin et al 1996). Modification of the urine matrix with ammonium nitrate did not completely abolish matrix interferences and an additional standardization procedure, entailing solid-phase extraction on strong cation exchange cartridges, was required. The method enables accurate measurements of trace lithium in plasma and urine at concentrations down to 0·03 μM with intra- and inter-day variability $<10\%$.

Whereas other investigators (Shen et al 1988; Bencze et al 1989; Miller et al 1989; Dürr et al 1990; Sampson 1991; Shalmi et al 1994) have also reported the use of electrothermal atomic-absorption spectroscopy for the quantitative determination of trace lithium in plasma and urine, to the best of our knowledge only one report has described direct measurement of trace lithium in erythrocytes (Genyuan et al 1995). In this study the mean concentration of trace lithium measured by electrothermal atomic-absorption spectroscopy in the erythrocytes of 33 healthy subjects was $1{\cdot}97 \pm 0{\cdot}22$ μM ($13{\cdot}7 \pm 1{\cdot}56$ $\mu g\,L^{-1}$). The instrument was calibrated with aqueous lithium solutions in the presence of mixtures of model cations (K^+, Na^+, Ca^{2+} and Mg^{2+}) at the levels assumed to be present in the erythrocytes. This approach, however, neglects other components that might interfere with the atomic-absorption response of lithium. Moreover, the results of recovery studies were inappropriately high because the quantity of lithium (200 $\mu g\,L^{-1}$) added to samples of erythrocyte supernatant was approximately ten times the concentration of lithium measured in red blood cells in their experiments (approx. 20 $\mu g\,L^{-1}$). Although electrothermal atomic-absorption spectroscopy should be sufficiently sensitive for quantification of lithium in red blood cells, our exploratory experiments showed that the lithium absorption signal suffers from severe matrix interferences, notably ionic cellular components, necessitating an additional optimization step compared with urine and plasma samples. Furthermore, the introduction in our laboratory of a new type of pyrocoated graphite tube with integrated platform necessitated reappraisal of the furnace temperature programme and rinsing procedure. We describe here the development of an electrothermal atomic-absorption spectrometric method enabling sensitive measurement of trace lithium concentrations in human erythrocytes.

Materials and Methods

Equipment

Atomic-absorption spectrometry was performed with a Perkin-Elmer (Überlingen, Germany) 1100B spectrophotometer equipped with an HGA-700 graphite furnace and an AS-70 autosampler. The newly designed tubes (B3001264), purchased from Perkin-Elmer, are machined from one piece of graphite and are totally pyrocoated. Their U-shaped platform accommodates up to fifty microlitres of sample. A lithium hollow-cathode lamp for the Perkin-Elmer instrument (Photron, Victoria, Australia) was operated at 15 mA with a 0·2-nm slit. The wavelength was set at 670·8 nm. The analytical conditions are given in Table 1. Argon was used as purge gas, at a flow rate of 300 mL min^{-1} (20 mL min^{-1} during atomization).

Reagents

Ammonium nitrate (MicroSelect $>99{\cdot}5\%$), and magnesium chloride ($>99{\cdot}9\%$) were purchased from Fluka Chemie (Buchs, Switzerland); trichloroacetic acid (TCA) 10% solution and Triton X-100 were from E. Merck (Darmstadt, Germany). Solutions of nitric acid (0·4 M) and hydrochloric acid (0·2 M) were prepared from concentrated acids (E. Merck). Lithium-free solutions were prepared with twice-distilled water and stored in polypropylene vials.

Li standard solutions were prepared by diluting a 1000 $\mu g\,mL^{-1}$ (0·144 mmol mL^{-1}) Li solution in 2% HCl (lithium atomic-absorption standard solution; Janssen Chimica, Beerse, Belgium) with twice-distilled water to give solutions ranging from 1 to 4 $\mu g\,L^{-1}$ (0·14–0·58 μM).

Sample preparation

As part of a study approved by the Ethics Committee of the Lausanne University Hospital, blood samples (10 mL) from psychiatric patients and healthy donors were collected in EDTA-K Monovettes (Sarstedt, Nümbrecht, Germany). Disposable plastic pipettes were used throughout sample processing to avoid contamination by lithium contained in glass. A portion of the sample (approx. 7 mL) was centrifuged for 10 min at 1000 *g* (2400 rev min^{-1}) and the plasma transferred into polypropylene tubes. The residual plasma and the buffy coat at the interface of the red cells were discarded. The packed erythrocytes (approx. 2 mL) were gently washed once with isotonic $MgCl_2$ (110 mM, 6 mL) for exactly 30 s and the erythrocyte suspension was centrifuged for 10 min at 1000 *g*. The rinsing solution was collected and after removal of the interface the washed erythrocytes were stored at −30°C before analysis.

Proteins and other solid cellular components were precipitated by addition of TCA (10%, 500 μL) and Triton X-100 (1%, 10 μL) to haemolysed red cells (250 μL; dilution factor 3·04). Samples were sonicated for 2 min and then centrifuged 10 min at 20 160 *g* (14 000 rev min^{-1}) at 4°C on a Hettich Universal 16R benchtop centrifuge (Bäch, Switzerland). The matrix of the red blood cell supernatant was modified by addition of NH_4NO_3 (10 M, 25 μL) and HNO_3 (0·4 M, 25 μL) to the processed samples (200 μL). Twice-distilled water (20 μL) was added to the resulting solution (25 μL) and the mixture was introduced into the graphite furnace.

Conditions used for electrothermal atomic-absorption spectroscopy

Furnace conditions were experimentally established (Table 2) to provide reproducible results with acceptable sensitivity and accuracy, while adequately removing the biological matrix. Optimum preconditioning (char II) and atomization temperatures were 1250°C and 2500°C, respectively. Compared with urine and plasma samples (Magnin et al 1996) it was necessary to increase substantially (> 200°C) the temperature of the char II step to remove intracellular ionic species which created considerable interferences (fumes) during the atomization step. The temperature of the char I step remained at 550°C whereas the drying times at 120 and 150°C had to be increased because of the design of the tubes with the U-shaped platform. Furthermore, the addition of 0·1% Triton X-100 to the rinsing solution (0·2 M HCl) used to wash the capillary tubing of the autosampler enabled homogenous sample addition and hence reproducible results during addition-calibration. Other specifications of the analysis of lithium in red blood cells were similar to those previously described (Magnin et al 1996); the absorption wavelength was set at 670·8 nm and deuterium background correction was not used at that high wavelength.

Calibration of the instrument

In the absence of lithium-free red blood cells, matrix-matched samples were used to establish calibration curves by the addition-calibration method. On each day of operation serial quantities of lithium were added to a previously prepared reference red blood cell extract pool. The parameters (slope and offset) of the established calibration were used for quantitative analysis of all subsequent samples.

All measurements were made in duplicate with the duplication process starting at the red blood cell processing step, to detect variability associated with the matrix-removal procedure.

The measured concentration of lithium in red blood cells was multiplied by a factor of 3·04 (760/250), to take into account the addition of TCA (500 μL) and Triton X-100 (10 μL) to the samples (250 μL). A further correcting factor (f = 1·13) for the residual volume of the washing solution ($MgCl_2$ 110 mM) trapped in red blood cells was also introduced.

Reference pool samples and internal quality controls

A large volume of reference pools of red blood cell extracts (obtained from healthy donors) was constituted, processed as previously described and stored as 1·0 mL samples at −20°C after addition of the matrix modifier. These samples were individually thawed on the day of analysis to establish the addition-calibration curves; the samples were also used as internal quality control samples throughout the analysis. They were analysed every five samples to establish intra-assay variation and on different days of analysis for determination of inter-day variation.

Recovery of lithium after extraction from haemolysed erythrocytes

Lithium solution (0·5, 0·75, 1·0 and 1·25 μg L^{-1}; 120 μL) was added to samples (250 μL) of haemolysed red blood cells. In contrast with previously reported experiments (Genyuan et al 1995) in which recovery experiments were performed with red blood cells to which 200 μg L^{-1} (28·8 μM) lithium had been added, the amount added was of the same order of magnitude as the lithium content presumably present in the biological samples. The

fortified samples were processed as described above and the trace lithium quantified. The results were compared with those obtained from haemolysed erythrocytes diluted similarly with 120 μL ultrapure water.

To determine if the matrix-modification procedure adequately suppressed interference with lithium atomization arising from matrix components, known volumes of 1 and 2 $\mu g L^{-1}$ lithium solutions were added to extracts of red blood cells containing NH_4NO_3 (10 M) and HNO_3 (0·4 M) (i.e. samples ready for analysis) and analysed directly by atomic-absorption spectrometry.

Assessment of the volume of rinsing solution trapped in samples of red blood cells

The amount of plasma (or any extracellular solution) trapped in red blood cells can be quantified, and therefore corrected for, by use of a variety of extracellular markers (for example CoEDTA, [^{131}I]albumin and [^{14}C]sucrose). We have developed a sensitive assay for quantification of sinistrin (Séchaud et al 1996), a polyfructosan related to inulin, the distribution of which is assumed to be essentially extracellular and which has already been used for this purpose (Evrard et al 1978). A series (n = 6) of samples (1·0 mL) of intact erythrocytes previously submitted to the rinsing procedure with $MgCl_2$ (110 mM) described above were gently mixed for 1 min with a known volume (0·5 mL) of sinistrin (70 $\mu g mL^{-1}$) dissolved in 0·9% NaCl. The suspension was centrifuged (10 min, 2400 rev min^{-1}, 4°C) and the supernatant collected. Both the original sinistrin solution and the supernatant (n = 6) were assayed for sinistrin content by HPLC. It was assumed that the volume of trapped extracellular solution is responsible for the dilution of the starting sinistrin solution and can be therefore determined by the classic formula $C_1 \times V_1 = C_2 \times V_2$. Thus, a factor could be estimated to correct red blood cell lithium levels for trapped rinsing solution. Results are expressed as mean ± s.d.

Results

Method development for the determination of trace lithium in erythrocytes

The presence of severe interference (fumes at the beginning of the atomization step), presumably from co-extracted salts, obliterated the lithium absorption signal and precluded the use of the procedure (matrix modification and atomic-absorption spectrometry programme) previously used for urine and plasma samples (Magnin et al 1996). Addition of the reported matrix modifier NH_4NO_3 (1·2 M) to the extract of red blood cells eliminated this effect only partially. To prevent the interferences, various matrix modifiers such as potassium hydrogen phosphate, nitric acid and others were tested. The addition of a mixture of 10 M NH_4NO_3 and 0·4 M HNO_3 (1 + 1; 50 μL) to the extract of red blood cells (200 μL) was found to be optimum for eliminating the interferences from the ionic species. A crucial step in the optimization was the changing of the preconditioning (char II) temperature from 1050°C to 1250°C. At this high temperature lithium chloride is volatile and the presence of concentrated nitrate salts is necessary to form the less volatile lithium nitrate. Furthermore, the design of the tube with the U-shaped integrated platform required a substantial increase in the time of the drying steps at 120°C and 150°C. The analytical conditions chosen for the quantitation of lithium in extracts of red blood cells are shown in Tables 1 and 2.

Calibration curves and limits of linearity

During the validation step, the concentration of lithium measured in the processed extracts of red blood cells never exceeded 3 $\mu g L^{-1}$ (0·43 μM). The addition-calibration obtained with extracts of red blood cells in the presence of NH_4NO_3 and HNO_3 was therefore established up to 4 $\mu g L^{-1}$ (0·58 μM) and found to be linear, with a coefficient of determination $1·0 > r^2 > 0·99$. During analysis of the study samples exceptionally high concentrations of lithium were found in some processed extracts of red blood cells; these were therefore diluted with the matrix-modifier solution to concentrations encompassed by the calibration range.

Accuracy and recovery

In the absence of lithium-free red blood cells, determination of the recovery of trace lithium from this biological medium reflects the accuracy of the

Table 1. Analytical conditions used for determination of trace lithium in red blood cells.

Wavelength	670·8 nm
Slit width	0·2 nm
Slit height	Low
Hollow-cathode lamp current	15 mA
Calibration	Addition-calibration
Standard	5, 10 and 15 μL of Li standard solution (4 $\mu g L^{-1}$)
Read delay	0·0 s
Integration time	8·0 s
Signal processing	Peak area
Sample volume	25 μL supernatant (containing 2·5 μL 10 M NH_4NO_3 + 2·5 μL 0·4 M HNO_3)
Background correction	Off

Table 2. Optimized furnace conditions for atomic-absorption spectrometry of erythrocyte extracts using pyrocoated graphite tubes with integrated platform.

Step	Temp (°C)	Ramp time (s)	Hold time (s)	Argon flow ($mL\,min^{-1}$)
Dry	120	5	60	300
Dry	150	5	40	300
Char I	550	10	30	300
Char II	1250	10	30	300
Cool	20	1	10	300
Atomization	2500	0	8	20
Clean	2650	1	5	300

method. The addition-calibration method was therefore used for the determination of the recovery and the accuracy of the analysis of trace lithium in red blood cells fortified with a known amount of lithium. Two types of experiment were performed, evaluation of the effect of the extraction procedure on the recovery of trace lithium from red blood cells and detection of any matrix effect which could interfere with the lithium signal during the atomization step.

Recovery of lithium after extraction from haemolysed erythrocytes

Lithium solution (0·5 to 1·25 $\mu g\,L^{-1}$, 120 μL) was added to samples (250 μL) of haemolysed red blood cells. Measured lithium levels were compared with those obtained from haemolysed erythrocytes diluted similarly with 120 μL ultrapure water (Table 3). The CV (%) of the measured concentrations never exceeded 10% and the accuracy (i.e. the recovery) was within 6% throughout the whole range of concentrations added to the cells. These results indicate that the extraction procedure is efficient, i.e. that no analyte is lost. The analytical method enables concentration differences as small as 0·25 $\mu g\,L^{-1}$ to be distinguished.

Recovery of lithium added to the erythrocytes extract pool

The matrix-modification procedure adequately suppressed interferences from co-extracted components on lithium atomization. Known volumes of 1 and 2 $\mu g\,L^{-1}$ lithium solution were added to red blood cell extracts containing NH_4NO_3 (10 M) and HNO_3 (0·4 M) (i.e. samples ready for atomic-absorption spectrometry); the analytical results are shown in Table 4. The concentrations measured in the control extract samples (concentrations in the extracts plus the amount added) never differed by more than 4% from those expected, suggesting that the matrix modifier added to red blood cell supernatant enables suppression of interferences likely to obscure the lithium signal during atomization. The extract fortified at the highest level (2·59 $\mu g\,L^{-1}$) corresponds to a concentration in erythrocytes of 7·87 $\mu g\,L^{-1}$ (1·13 μM) taking into account the dilution by a factor of 3·04 which occurred before analysis. In most of the clinical samples collected in this study the concentrations of trace lithium in

Table 3. Recovery of trace lithium added to haemolysed erythrocytes.

Sample	n	Measured concn ($\mu g\,L^{-1}$)	s.d.	CV (%)	Concn ($\mu g\,L^{-1}$) in red blood cells (dilution factor = 3·04)	Theoretical concn ($\mu g\,L^{-1}$)	Accuracy (%)
112B-89A3 RBC (250 μL) + water (120 μL)	14	0·489	0·047	9·70	1·487	1·487	—
112B-89B3 RBC (250 μL) + lithium (0·5 $\mu g\,L^{-1}$ in 120 μL)	7	0·619	0·037	6·01	1·880	1·987	−5·34
112B-89C3 RBC (250 μL) + lithium (0·75 $\mu g\,L^{-1}$ in 120 μL)	7	0·727	0·048	6·63	2·211	2·237	−1·17
112B-89D3 RBC (250 μL) + lithium (1·0 $\mu g\,L^{-1}$ in 120 μL)	7	0·826	0·038	4·63	2·510	2·487	0·95
112B-89E3 RBC (250 μL) + lithium (1·25 $\mu g\,L^{-1}$ in 120 μL)	7	0·896	0·04	4·51	2·723	2·737	−0·50

RBC = red blood cells. Accuracy % = (measured—theoretical)/theoretical.

Table 4. Recovery of lithium added to erythrocytes extract pool.

Sample	n	Measured concn ($\mu g\,L^{-1}$)	s.d.	CV (%)	Theoretical concn ($\mu g\,L^{-1}$)	Accuracy (%)
112B-87 RBC extract (25 μL) + water (20 μL)	7	0·586	0·053	9·03	0·586	—
112B-87 RBC extract (25 μL) + lithium (1·0 $\mu g\,L^{-1}$ in 20 μL)	6	1·632	0·118	7·26	1·586	2·90
112B-87 RBC extract (25 μL) + lithium (2·0 $\mu g\,L^{-1}$ in 20 μ L)	5	2·488	0·142	5·71	2·586	−3·79

RBC = red blood cells. Accuracy % = (measured − theoretical)/theoretical.

the red blood cells were below this value. However, in some exceptional cases the concentrations of trace lithium encountered were higher but could nevertheless be adequately quantified after dilution with the matrix-modifying solution.

Intra-day variation

The red blood cell extract pool analysed every five samples was used as a quality-control sample for the determination of the intra-assay variability. The mean intra-assay precision (CV%) of the analysis of the extract pool was 11·4% in series of no more than 40 samples analysed in duplicate. After approximately 80 firings a solid charcoal-like residue was observed on the platform of the graphite tubes and a notable drift in the concentration measured in the extract control pool was seen, with poor CV% of duplicate analyses thereafter. It was therefore necessary to use a new pyrocoated graphite tube each day of analysis and to arrange series containing fewer than 80 consecutive analyses to achieve acceptable (CV better than ±15%) within-day variability.

Inter-day variation

The mean lithium concentration in the erythrocytes extract pool used for internal quality control was $0{\cdot}46 \pm 0{\cdot}04\,\mu g\,L^{-1}$ with 8·4% inter-day (inter-analysis) variation. The evolution over three weeks of the lithium concentrations of the reference red blood cells extract pool is shown in Figure 1. Taking into account the dilution factor of 3·04, this corresponds to a concentration of $1{\cdot}40 \pm 0{\cdot}12\,\mu g\,L^{-1}$ ($0{\cdot}20 \pm 0{\cdot}02\,\mu M$) in red blood cells. The factor correcting for contamination of red blood cells by rinsing solution was not used in this calculation. The mean slope of the standard addition calibration established with the red blood cells extract pool was $0{\cdot}031 \pm 0{\cdot}004$ (n = 22), which is lower than the values of $0{\cdot}036 \pm 0{\cdot}002$ and $0{\cdot}037 \pm 0{\cdot}004$ established for plasma and urine, respectively, albeit with a different temperature programme for the red blood cell matrix including a char II step at 1250°C instead of the 1050°C previously used for plasma and urine matrices (Magnin et al 1996).

Interestingly, the inter-day variation of lithium concentration in the reference red blood cells extract pool was somewhat smaller than the within-assay precision (CV% 8·4 compared with 11·4), demonstrating that the inter-assay drift of the values of the internal quality-control pool was acceptable, but stressing the importance of changing the pyrocoated graphite tube each day and analysing series of fewer samples (< 40 in duplicate).

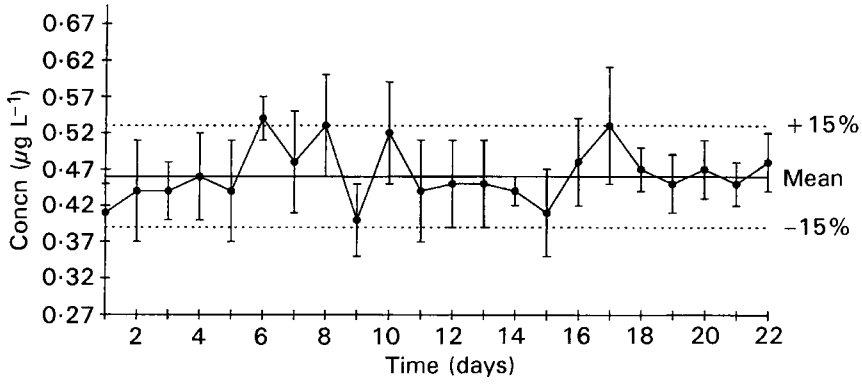

Figure 1. Evolution over 22 days of the measured lithium concentration of a reference erythrocyte extract pool (mean concentration $0{\cdot}46 \pm 0{\cdot}04\,\mu g\,L^{-1}$). Each data point is the mean (± s.d.) of the results of determinations performed after every five samples on the same day.

The CV% values obtained for the intra- and inter-assays were, therefore, lower than the value (15%) recommended by the Conference Report on Bioanalytical Validation (Shah et al 1992).

Assessment of the volume of the washing solution trapped in samples of red blood cells

Assuming that sinistrin is accumulated in the extracellular fluid only, the mean volume of dilution of a 500-μL sample of sinistrin solution added to 1 mL washed erythrocytes was 612 ± 27 μL ($n = 6$). The difference corresponds to the volume of extracellular fluid and represents $11{\cdot}2 \pm 2{\cdot}7\%$ of the total volume of the erythrocyte sample. Therefore, the concentrations of trace lithium measured in red blood cells by atomic-absorption spectrometry should be corrected by the factor $100/(100-11{\cdot}2) = 1{\cdot}13$ to give the actual intracellular level of trace lithium.

Trace lithium levels in plasma and red blood cells from healthy subjects

Levels of trace lithium were measured in the plasma and red blood cells from 40 healthy subjects devoid of psychiatric and somatic history (21 males; median age 27, range 19–53; median weight 66 kg, range 45–93).

The median level of lithium in plasma was 0·13 μM (0·90 $\mu g\,L^{-1}$), the inter-quartile range was 0·11 to 0·19 μM (0·76–1·32 $\mu g\,L^{-1}$) and the total range was 0·08 to 12·2 μM (0·56–84·7 $\mu g\,L^{-1}$). The median level of lithium in red blood cells was 0·23 μM (1·60 $\mu g\,L^{-1}$), the inter-quartile range was 0·20 to 0·30 μM (1·39–2·08 $\mu g\,L^{-1}$) and the total range was 0·14 to 5·0 μM (0·97–34·7 $\mu g\,L^{-1}$). Both variables were highly correlated ($r = 0{\cdot}91$, $P < 0{\cdot}0001$). The median value of the red blood cells/plasma ratio was 1·57, with an inter-quartile range of 1·16 to 2·07 and extreme values of 0·21 and 2·7. The distribution of the values seems to be highly skewed, because of some high outliers. One common factor was found a-posteriori in the six subjects with the highest plasma and red blood cell lithium levels—they were all recruited from volunteers submitted to a controlled diet as a preparation to physiological investigations and had been given Vichy Célestins water during preceding days. This water is known for its high lithium content. Interestingly, these subjects also had mean red blood cell/plasma ratios lower than those of the other subjects (0·39 compared with 1·77, $P < 0{\cdot}0001$). These six outliers had a minimal influence on the medians and inter-quartile ranges of trace lithium reported above.

Discussion

To prevent efflux of lithium from red blood cells via the Li–Na counter-flow system (Ostrow et al 1978; Ehrlich & Diamond 1980) during washing of the erythrocytes with a solution free from trace lithium, it was essential that lithium was not exchanged by counter-transport mechanisms implicating extracellular sodium ions. The isotonic 110 mM $MgCl_2$ solution proposed by other authors for rinsing red blood cells (Szentistvanyi et al 1980; Rybakowski et al 1981), was therefore chosen for washing of the erythrocyte suspension for a 30-s period. No trace lithium could be detected in subsequent rinsing solution. The volume of residual $MgCl_2$ solution trapped in the erythrocyte pellet must be taken into account in the final calculation of the intra-cellular concentration of trace lithium. This washing solution increased the volume of the red blood cells by $11{\cdot}2 \pm 2{\cdot}7\%$, a value very similar to the $11{\cdot}1 \pm 0{\cdot}4\%$ reported for the plasma contamination of unwashed red blood cells determined by [^{14}C]inulin dilution (Evrard et al 1978).

Trace lithium in erythrocytes can be measured by electrothermal atomic-absorption spectroscopy with the required sensitivity and accuracy after extraction and the matrix-modifying treatment. The so-called tubes with the U-shaped integrated platform, first introduced with the transversely heated graphite furnace and available since 1996 for the longitudinally heated graphite furnace, have many advantages over the traditional L'Vov platform (Schlemmer 1996). In our experiments the lithium absorption signal was sharper because the pyro-coated graphite tubes are machined in one piece, thus reducing the lag time necessary for the temperature to become uniform throughout the platform. The new tubes thus enable appreciable reduction of the atomization time from 10 to 8 s. Furthermore, the U-shape of the integrated platform enables the use of a larger volume and prevents movement of the sample on the tube wall, as has occasionally been observed with the traditional L'Vov platform.

This highly precise and sensitive method enables differentiation between samples differing in lithium concentrations by at least 0·25 $\mu g\,L^{-1}$ (0·04 μM). The analysis of samples from healthy Caucasian subjects ($n = 40$) suggest that the range of concentrations of trace lithium in erythrocytes was more variable and lower than previously reported in a healthy Asian population ($1{\cdot}97 \pm 0{\cdot}22$ μM ($13{\cdot}7 \pm 1{\cdot}56$ $\mu g\,L^{-1}$) (Genyuan et al 1995) comprising fewer subjects ($n = 33$). It is difficult to attribute this difference either to population differences (lithium content of diet, genetic influence) or to the analytical procedure used. We observed that intake of mineral water (Vichy Célestins) with naturally high concentrations of trace lithium (3·4 $mg\,L^{-1}$) by Caucasian subjects on a

standardized diet (see above) resulted in increased levels of lithium in their urine, plasma and erythrocytes. This finding implies that dietary factors have a substantial effect. On the other hand, from an analytical standpoint, Genuyan et al (1995) used aqueous lithium concentrations in the presence of a model mixture of ions (Na^+, K^+, Ca^{2+}, Mg^{2+}) to establish calibration curves. They reported that standard-addition to erythrocyte extracts or the use of model mixtures gave a similar calibration. In our hands, the slopes of the plots obtained with an aqueous solution were substantially different from those obtained for biological matrices. The effects of the biological matrices, although controlled by the addition of a matrix modifier, were not completely abolished.

For most subjects we found a red blood cell/plasma ratio > 1, implying that trace lithium accumulates in erythrocytes. This finding is not in accord with average values of approximately 0·5–0·8 reported in most studies of red blood cell/plasma ratios measured during lithium treatment (Ostrow et al 1978; Ramsey et al 1979; Ehrlich & Diamond 1980; Motas de Freitas et al 1991). Interestingly, our subjects with high intake related to mineral water had significantly lower red blood cell/plasma ratios. This might indicate that the lithium equilibrium across the erythrocyte membrane is different for trace and higher concentrations (involvement of supplemental transport mechanisms with low capacity, limited number of high-affinity intracellular binding sites?). The kinetics of trans-membrane exchanges could also play some role (delayed equilibrium?).

In combination with the recently published quantitation of trace lithium in plasma (Magnin et al 1996), the current report makes it possible to determine for the first time the red blood cell/plasma ratio for trace levels of lithium. Because neither levels of trace lithium in red blood cells nor the red blood cell/plasma ratio have ever been considered in psychiatric evaluation (Dawson 1991), we are currently applying the proposed atomic-absorption spectrometry method to the analysis of samples collected in a study of the connection between trace lithium levels and psycho-affective disorders.

In conclusion, careful adaptation of the previously published method was necessary for measurement of trace lithium in erythrocytes. The procedure we report has the sensitivity and accuracy required for determination of trace lithium in red blood cells, and might prove of invaluable interest as an investigational tool in epidemiological studies assessing exposure to environmental lithium in the diet and beverages, inter-individual variations in trans-membrane and renal lithium kinetics, and possible relationships with pathological conditions such as psycho-affective disorders.

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