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New direct method for measuring red cell lithium

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SUMMARY A new direct method for the measurement of red cell lithium was compared with the indirect method. Good correlation (r = 0·97) was found and the coefficients of variation of the direct and indirect methods were 3·9% and 5·5%, respectively. In the direct method red cells suspended in choline chloride were centrifuged through dibutyl phthalate, which removes plasma adherent to the cells. A haemolysate is made of the sedimented red cells. The lithium concentration of this was measured by atomic absorption spectrophotometry.

There are many conflicting reports about the value of red cell lithium, and methodological problems have been proposed as a reason for this. It is suggested that the simpler, more precise direct method described here should be used in future.

Lithium is widely used in the treatment of affective disorders. The value of measuring serum lithium concentrations in patients to aid with dose adjustments and diagnosis of toxic symptoms has been known for many years. The role of intracellular lithium measurements is more controversial.

Frazer *et al* suggested that red cell lithium concentrations may correlate better with those in the brain than plasma lithium concentrations.[1] This would explain the findings of several groups[1–4] that patients who show a good response to lithium have higher red cell lithium concentrations and higher lithium ratios (red cell lithium: plasma lithium) than those who do not respond. These results suggest a possible use of intracellular concentrations as a predictor of response to lithium. Other groups, however, have been unable to confirm this.[5–7] It has also been shown that patients with bipolar affective disorders have higher lithium ratios than those with unipolar disease,[8] although this too has been disputed.[5]

There are suggestions that red cell lithium may sometimes be a better indicator of lithium toxicity than plasma concentration. Elizur *et al* showed that patients with symptoms of lithium toxicity had significantly higher lithium ratios than did those without toxic symptoms.[9] The plasma lithium concentrations were not significantly different. Indeed, most patients exhibiting toxic symptoms had plasma lithium concentrations within the therapeutic range. Similar findings have been reported by Hewick *et al*.[10]

Both direct and indirect methods of measuring red cell lithium have been used.[3 5 11] Frazer *et al* have criticised the indirect method for being much more variable and less accurate than a direct method, and suggest that some of the conflicting results may be explained by methodological differences.[12]

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Material and methods

The direct method used was an adaptation of the simple micromethod for the determination of erythrocyte electrolyte concentrations described by Suzuki.[13] The formula used to calculate the red cell lithium by the indirect method was:

$$\text{Red cell lithium} = \text{whole blood [lithium]} - \frac{(1\text{-haematocrit}) \times \text{plasma [lithium]}}{\text{Haematocrit}}$$

Patients receiving lithium were recruited as volunteers to the study from a lithium clinic, with approval of the ethical committee. Thirty four samples were obtained from 33 patients: two samples from one patient were taken three weeks apart. Venous blood was collected from each patient into two Vacutainer tubes (Becton Dickinson) containing edetic acid anticoagulant. Blood from one tube was used for the determination of the haematocrit by the Coulter Counter S Plus. The 5–6 ml of blood in the second tube were well mixed and 99 μl were diluted 1/20 with distilled, deionised water, using a Hamilton digital dilutor. The remaining blood was then spun at 1600 × *g* for 10 minutes and the plasma removed by aspiration. A 1/20 dilution in water was made of 99 μl of plasma.

For the direct method, 200 μl of the remaining packed red cells were dispersed into 1 ml of 150 mM choline chloride (Sigma Chemical Company), which was layered on 0·2 ml of dibutyl phthlate in a 1·5 ml microfuge tube. The samples were immediately centrifuged at 8800 × g for two minutes. Dibutyl phthlate has a density between that of water and erythrocytes. The erythrocytes were therefore sedimented to the bottom of the tube, passage through the dibutyl phthlate removing the adherent plasma. A 1/20 dilution of the packed cells was made and mixed thoroughly to ensure complete haemolysis.

Lithium concentrations were measured on the dilutions of packed cells, and on whole blood and plasma dilutions for the indirect method, using an Instrumentation Laboratory AA/AE spectrophotometer. Each assay on each sample was performed in duplicate, and paired results for each method were therefore obtained.

Results

Fig 1 is a scatter diagram showing the correlation between the new direct method and the indirect method. The averages of the paired results for each method were used. The regression equation, calculated by Deming's method[14] is:
$y = 0{\cdot}9905x - 0{\cdot}0084$ (where x = indirect method result and y = direct method result).
The correlation coefficient was 0·97.

An alternative approach to assessing any difference between the two methods is to use an Altman-Bland plot (fig 2).[15] This shows an overall negative bias of

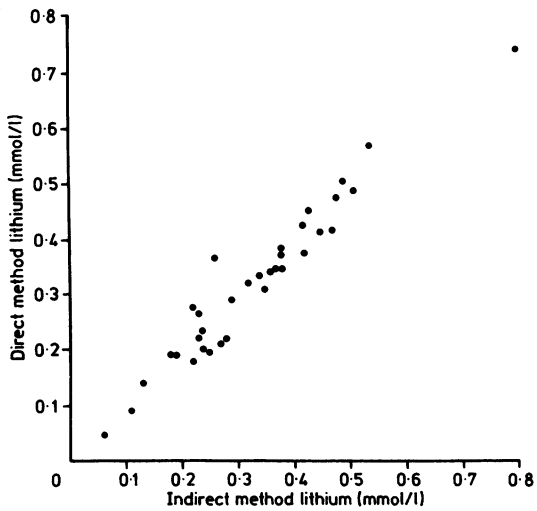

Fig 1 *Scatter diagram showing correlation between direct and indirect methods for measurement of red cell lithium, using the means of paired results for each method.*

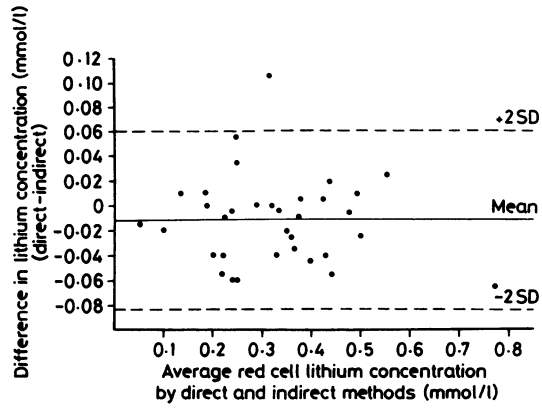

Fig 2 *Altman-Bland plot showing mean (± 2 SD) difference between red cell lithium concentrations, measured by direct and indirect methods, plotted against mean red cell lithium concentration measured by both methods.*

0·012 mmol/l for the direct method compared with the indirect method. The ±2 SD (~95%) limits of agreement were −0·084 to +0·060, range of 0·144 mmol/l.

Analytical variance was calculated for each method by the method of paired replicates using the formula $SD^2 = \Sigma d^2/2n$, where d is the difference between duplicates, and n is the number of paired results. The coefficients of variation (CV) for the methods were as follows: plasma lithium 0·9%; direct red cell lithium 3·9%; indirect red cell lithium 5·5%. These were calculated using the overall mean for each method, and therefore apply to the whole range of concentrations covered by the samples that were analysed.

Discussion

Lithium has been measured directly on haemolysates of packed red cells after centrifugation of whole blood.[16] As the lithium concentration in the plasma may be several times higher than that within the red cells, however, plasma trapping causes the measurement to be artefactually high.

Plasma trapping can be quantified and therefore corrected by using a variety of extracellular markers including Cobalt edetic acid,[11] ^{131}I-human serum albumin,[17] inulin,[17] and ^{14}C-sucrose.[18] The fraction of plasma trapping found using these markers seems to depend on the molecular weight of the compound and ranges from about 1% to 4%. These methods are time consuming and require facilities and equipment not readily available in all laboratories.

Suzuki *et al*, using ^{3}H-inulin, found that plasma trapping was reduced to less than 0·3% when red cells were spun through dibutyl phthalate.[13] This level of plasma trapping has an insignificant effect on the

measurement of lithium in haemolysates of packed red cells. We found the precision of the method to be good with a CV of 3·9%. This is higher than the CV of the plasma lithium measurements, presumably because of the high viscosity of the packed red cells.

Frazer *et al* suggested that the indirect method gives highly variable results because it depends on a calculated small difference between two larger values.[12] Our assessment of the precision of this method did not confirm this. The CV was 5·5%. We found good correlation between the two methods, and the Altman-Bland plot showed only a slight negative bias of the new direct method compared with the indirect method.

Our method has the advantage of being quick and easy to perform and can be done within most clinical chemistry laboratories which are already measuring serum lithium. We suggest that future studies on the relevance of red cell lithium concentrations should be done using this simple, precise method.

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