J. Pharm. Pharmac., 1978, 30, 683–685 Received May 25, 1978

Lithium-6 stable isotope determination by atomic absorption spectroscopy and its application to pharmacokinetic studies in man

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No useful radioisotope of lithium exists to assist in the study of its biochemical pharmacology. A simple method has been developed for the determination of the stable isotope ^{6}Li by atomic absorption spectroscopy (AAS). The technique is applicable in any laboratory equipped with simple AAS apparatus. Analysis of total lithium content (E) by flame emission spectroscopy is followed by separate determination (A_6 and A_7) of atomic absorption by the sample of the light emitted by separate hollow cathode lamps manufactured from the isotopes ^{6}Li and ^{7}Li. The standard curve of absorption ratio (A_6/A_7) against isotopic ratio (^{6}Li/^{7}Li) at any concentration (E) is an exponential which may be solved using a simple programmable calculator. Application of this method to the study of the pharmacokinetics of ^{6}Li adminstered to 4 normal volunteers previously loaded with ^{7}Li suggests that the rate of appearance of lithium in blood is unaffected by the previous state of lithium loading.

Lithium is used in the prophylaxis of manic-depressive psychoses. Its mode of action remains obscure (Schou, 1976; Bunney & Murphy, 1976; Birch, 1978). One factor that has hindered the study of lithium in biological systems is that there is no suitable radioactive isotope: the isotopes ^{5}Li, ^{8}Li and ^{9}Li have half-lives of respectively 10^{-21}, 0·8 and 0·2 s. However the stable isotope ^{6}Li is readily available since it has been used in nuclear technology and recently was used to localize lithium in brain by autoradiography using neutron activation in a nuclear pile (Thellier, Steltz & Wissocq, 1976).

Stable isotope technology usually requires the use of mass spectrometry and such studies are possible in biological systems with ^{6}Li (Garrec, Jourdan & others, 1977). However, these techniques require expensive equipment and involve much time in sample preparation and analysis. We have therefore developed a technique, using atomic absorption spectrometry (AAS), whereby large numbers of ^{6}Li determinations in biological samples can be carried out with minimal preparation by any laboratory with fairly elementary AAS equipment.

METHODS

Theoretical basis

Our method is based on that of Wheat (1971) for the determination of isotope ratio in nuclear reactor materials which takes advantage of the coincidence in the spectra of the two naturally occurring isotopes ^{6}Li and ^{7}Li. The isotopic shift from ^{7}Li to ^{6}Li is 0·015 nm which is well below the resolution of conventional AAS. However, both ^{7}Li and ^{6}Li lines at 670·8 nm are doublets, also of separation 0·015 nm. Naturally occurring lithium (7% ^{6}Li, 93% ^{7}Li) thus has an apparent spectral line which is a triplet (Fig. 1).

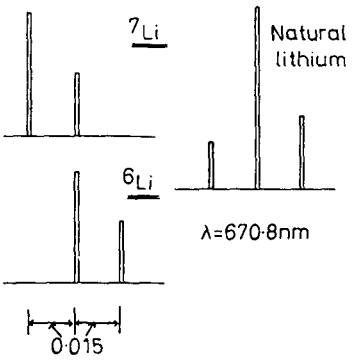

FIG. 1. Representation of the resonance line for lithium isotopes 6 and 7 at 670·8 nm, showing the triplet observed with 'natural lithium'.

Light from a hollow cathode lamp made from the element under analysis is absorbed by samples of atoms of the element introduced into the optical path. Therefore, two hollow cathode lamps are used, one made of ^{7}Li and one of ^{6}Li, and the absorbance ratio (A_6/A_7) determined, the triplet of 'natural lithium' may be resolved since the two isotopes absorb

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differentially the component fine lines of the triplet (Wheat, 1971). The calibration is exponential and a family of curves must be generated at a range of total lithium concentrations which are determined separately by flame emission spectrometry. Fig. 2(a) shows the absorbance of both ^{6}Li and ^{7}Li with both the ^{6}Li and ^{7}Li hollow cathode lamps, while Fig. 2(b) shows a typical calibration curve of (A_6/A_7) absorbance ratio against ^{6}Li/^{7}Li atomic ratio for different total lithium concentrations.

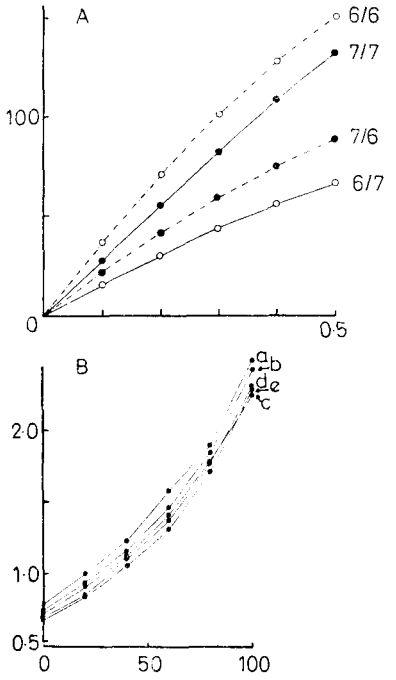

FIG. 2. (A) Absorbance under the same instrumental conditions of ^{6}Li and ^{7}Li using ^{6}Li or ^{7}Li hollow cathode lamps. Superscripts refer to the element determined, the subscripts to the lamp used. Ordinate: Absorbance (mm deflection). Abscissa: Lithium (mmol litre^{-1}). (B) Absorbance ratio determined for a range of isotopic enrichments at various total lithium concentrations. a 0·1; b 0·2; c 0·3; d 0·4; e 0·5 mmol litre^{-1}. Ordinate: Absorbance ratio. Abscissa: % ^{6}Li.

(a) *Analytical technique.* A Unicam SP90 Series 2, single beam AAS instrument was used after the conditions had been optimized for the various determinations. For ^{7}Li a standard Activion lithium lamp was used, the second source being a Westinghouse ^{6}Li lamp. The estimation of an unknown was carried out by three determinations of the sample (E,A_6,A_7). The total lithium content of the sample was first determined (E) by the SP90 in the flame emission mode and the sample was then diluted to within the concentration range of 0·01 to 0·09 mmol litre^{-1}. Using standards of total lithium concentration equal to that of the diluted unknown solution, the absorbance ratio (A_6/A_7) was determined with the SP90 in the Absorption mode using a range of isotopic ratios (^{6}Li/^{7}Li). The absorbance of each standard was determined once using the ^{6}Li lamp ($=A_6$) and once with the ^{7}Li lamp ($= A_7$). Using a Hewlett-Packard programmable calculator (HP25) and the manufacturer's standard program, the absorbance ratio (y) and isotopic ratio (x) were fitted to an exponential curve by the equation $y = a.\exp(bx)$, and the a and b parameters were determined. The absorbance A_6 and A_7 of the unknown sample were then determined using the ^{6}Li and ^{7}Li lamps. The absorbance ratio (A_6/A_7) of the unknown was thus derived and the isotopic ratio was calculated by substitution of the a and b parameters from the standard curve into the exponential equation. The concentration range 0·01 to 0·09 mmol l^{-1} was chosen since the variation of the a parameter was found to be small in this range.

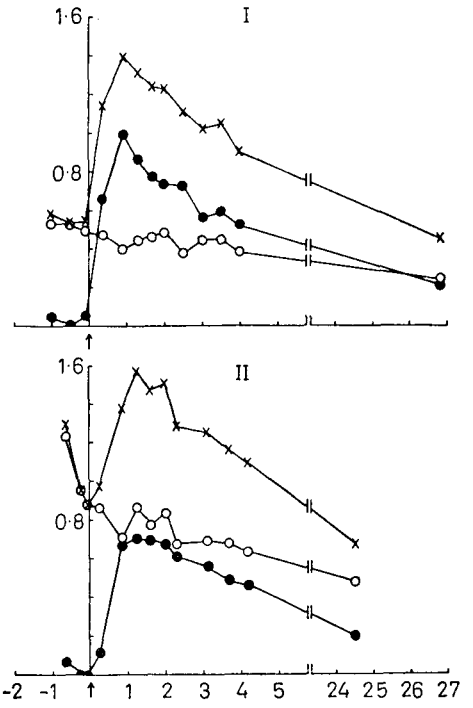

FIG. 3. Typical serum lithium profiles in normal male volunteers before and after the administration of 750 mg $^6Li_2CO_3$ at zero time (indicated by arrow) after 4 days administration of 'natural' Li_2CO_3 (250 mg three times daily). Total lithium content is represented by crosses, ^{6}Li by closed circles and ^{7}Li by open circles. Ordinate: Serum lithium (mmol litre^{-1}). Abscissa: Time (h).

Examples of the coefficients of variation for 10 estimations each of a set of standards of total lithium concentration 0·05 mmol litre^{-1} were 10·5% ^{6}Li, c.v. = 5·73%; 16·2% ^{6}Li, c.v. = 5·16%; 24·6% ^{6}Li, c.v. = 4·63%; 51·0% ^{6}Li, c.v. = 5·76%; 77·4% ^{6}Li, c.v. = 4·95%.

(b) *Pharmacokinetic studies.* Four normal volunteers were used to investigate the pharmacokinetics of a single oral dose of 750 mg of 95% enriched $^6Li_2CO_3$ taken after four days pretreatment with lithium carbonate B.P. (Camcolit, 250 mg three times daily). Blood was taken frequently by means of an indwelling venous cannula and the protocol was similar to that previously described for pharmacokinetic studies in man (Tyrer, Hullin & others, 1976).

RESULTS

Although there is individual sample error in the determination of the two isotopes, particularly at low enrichment and low concentrations, there is little immediate effect of a dose of ^{6}Li on the decline in serum ^{7}Li following the previous dose of lithium carbonate containing the naturally occurring isotopes (Fig. 3). The slope of the total lithium curve before zero time is continuous with that of ^{7}Li after the administration of the $^6Li_2CO_3$. The slope of the ^{6}Li curve is independent of the shape of the ^{7}Li curve and of the concentration of total serum lithium at the time of the ^{6}Li dose. For example, subject II received $^6Li_2CO_3$ only 2 h after a $^7Li_2CO_3$ dose and this is reflected in the steepness of the fall in serum ^{7}Li concentration in the initial stages. This suggests that the rapid absorption of lithium from the gut is not affected by the state of previous loading.

DISCUSSION

This method offers the possibility of fully investigating the pharmacokinetics of lithium doses in subjects currently receiving lithium. Obviously, it also has applications in other disciplines. Present generation AAS equipment should allow the method to be exploited at concentrations at least four times lower than those used here, thus making it feasible, for example, to determine two-way fluxes across red cell membranes at lithium concentrations which occur in patients receiving prophylactic lithium.

Acknowledgement

We thank Dr M. J. Tidd and Mr M. Scott for collaboration and discussions in the early stages of this project which was supported by the Medical Research Council.

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