BioMetals **12:** 375–381, 1999.

Effect of lithium augmentation on the trace elemental profile in diabetic rats

Devinder Dhawan[1], Ajaib Singh[1], Baljinder Singh[2], Harish K. Bandhu[1], Bakshish Chand[1] & Nirmal Singh[1]

[1]*Department of Biophysics, Department of Physics, Panjab University, Chandigarh 160 014, India (Fax: 91-0172-541409; E-mail: shahi%phys@puniv.chd.nic.in)*

[2]*Department of Nuclear Medicine, Postgraduate Institute of Medical Education & Research, Chandigarh 160 012, India*

Received 11 October 1999; accepted 22 October 1999

Key words: lithium, diabetes, trace elements, X-ray fluorescence

Abstract

Energy dispersive X-ray fluorescence technique was employed to study the interactions among various elements, viz.: K, Zn, Br, Fe, Cu, Br & Rb in 4 groups of rats viz. control-GI, diabetic- GII (diabetes induced by i.p administration of alloxan monohydrate at a dose level of 150 mg/kg b.w; single injection), lithium treated-GIII (lithium administered as Li_2 Co_3 at a dose level of 1.1 g of Li_2 Co_3/kg animal diet; free access; serum lithium levels – 0.5–1.2 mEq/L) and lithium + diabetic rats-GIV. The different treatments continued for a total duration of 1 month. The K contents were found to be significantly lowered in all the treatment groups which was maximum (28%) in lithium treated diabetic rats. Depression in the levels of Rb was noticed in lithium treated and lithium treated diabetic (G-III and G-IV) groups. However, the levels (Rb) remained unaltered in diabetic (G-II) group. Interestingly, a significant decline was observed in Fe levels in G-III following lithium administration but the levels remained unchanged in G-IV with lithium administration to diabetic rats. On the other hand, lithium treatment to normal (G-III) and diabetic (G-IV) rats indicated statistically significant elevation in the levels of Cu and Br. However, diabetic (G-II) rats did not indicate any elevation in the levels of these two elements. Interestingly, the concentrations of Zn were found to be significantly elevated in all the treatment groups, which was maximum (37%) in G-III (lithium) group. A comparison of various elements from lithium treated diabetic group G- IV with the corresponding elements from the diabetic group G-II, implied a significant depression in K and Rb levels and a significant elevation in the levels of Br.

Introduction

The usefulness of lithium (Li) as a potential remedy to treat various mental disorders effectively has been indicated in many previous studies (Pai *et al.* 1986; Lieff & Hermann 1989; Monking 1989). It is one of the very few cations that are tolerated in concentrations up to and above 1 m mol/kg body weight (Plenge 1970). Li, in general has not been considered as a hepatotoxic element (Schou 1986; Jefforson & Griest 1987). The support for Li not being a hepatotoxic element comes from the available reports on Li intoxication which makes no mention of hepatic dysfunction (El-Mallakh 1986; Amdisen 1988; Gadallah 1988). However, animal studies have shown variable results due to variations in experimental species and Li dosages.

Birch and Jenner (1973) have reported interactions between Li and Na^+/K^+ions in blood and other tissues. Li has been described by Schrauzer *et al.* (1992) to interact with other trace elements. We have reported (Singh *et al.* 1994) alterations in the levels of essential and non-essential elements in rat blood following Li administration. In a recent report (Singh *et al.* 1998) , it is indicated that Li augmentation

in affective disorder patients caused significant alterations in the concentrations of various elements (Fe, Cu, Zn, K, Ti) . On the other hand, alterations in the hepatic and renal levels of Cu, Zn, Fe, Mn and mg has been reported (Hare *et al.* 1985) in the experimentally induced diabetes.

It is known that the liver, intestine and kidney perform important function in the short-term regulation of trace element metabolism (Failla & Kisser 1983). Further, trace metals serve as co-factors for enzymes in numerous metabolic pathways and therefore, early variations in their levels reflect on the sequence of events required for biochemical adaptation to the stressed state. It has also been observed that diabetes like conditions may result from deficiency of certain trace metals (Arquilla *et al.* 1978).

Since, the biological interactions among the trace elements are so influential that nutritional and toxicological studies carried out with single elements might project inconclusive picture unless the levels of interacting elements in biological tissues are known.

In view of certain reports (Gershon 1970; Schou 1976; Mendels *et al.* 1981; Nyfeler *et al.* 1981; Haugaard *et al.* 1974; Rossetti 1989) emphasizing the insulin like action of Li and lack of information regarding its interactions with other metal ions in diabetic condition, the present study has been conducted to explore the role of Li treatment on the status of essential and non-essential elements in the liver of diabetic rats using energy Dispersive x-ray fluorescence (EDXRF) technique.

Experimental

Female Sprague Dauwley rats in the weight range of 130–150 g were procured from the central animal house of Panjab University, Chandigarh, India and were adapted in the Departmental animal house for one week prior to subjecting them to various treatments. The animals were randomly distributed in to four groups viz., control group-GI, diabetic group-GII , Li treated group-GIII and Li treated diabetic group – GIV.

Dosing

Li was administered to rats of groups GIII and GIV as Li carbonate Li_2Co_3in the powdered diet diet (Hindustan Lever Ltd. Mumbai, India) as slurry. Slurry was prepared by adding tap water to the powdered diet uniformally mixed with 1.1 g of Li_2Co_3/kg diet (dry weight). Li treatment continued for a period of one month and the rats had free access to diet and water throughout the study.

Diabetes was induced in rats of groups G-II and G-IV by giving single intraperitoneal injections of freshly prepared alloxan monohydrate (BDH Chemicals, UK) at a dose level of 150 mg/kg body weight. The diabetic state was supervised by estimating blood glucose levels intermittent weekly intervals and remained steady (with glucose levles in the range 100–200 mg/dl) for a period of one month.

Sample preparation

Animals of all the four groups (6–8 animals from each group) were sacrificed under light ether anesthesia after different treatments for a period of one month. Liver was excised and perfused with physiological saline to remove the blood traces completely and Livers were completely oven dried at 70 ^{0}C to a steady weight and grounded in an Agate pestle and mortar. The moisturefree samples thus obtained were weighed and mixed with equal amount of cellulose (Sigma Chemicals, USA). The cellulose was used as a binding agent. The sample cellulose mixture was then molded in to self-supporting pellets of 1 inch diameter by using a pure steel die and a hydraulic press (Paul Auto Weber, Germany). A constant and uniform pressure of 1055 kg/cm^{-2} was applied to the die head so as to get pellets of uniform thickness (75 mg cm^{-2}) below the critical thickness (Kumar *et al.* 1989; Dhawan *et al.* 1995; Bandhu *et al.* 1996).

Elemental analysis using EDXRF technique

The elemental analysis of liver samples in the form of pellets was carried out using EDXRF spectrometer involving a low power (100 W) tungsten anode X-ray tube (Kevex Inc. USA; 50 kV; 2.0 mA; water cooled) as a source of excitation with triaxial geometry and the experimental details are discussed elsewhere (Singh *et al.* 1998) . The triaxial geometry was used to minimize the background-scattered radiations. The X-ray tube was operated at 35 kV and 1.7 mA. The X-rays from the tube were made to fall on Molybdenum (Mo) secondary exciter and the characteristic K-X-rays of Mo (17.8 KeV, the weighted average energy of the Mo K_α and K_β lines) were in turn used to excite the characteristic X-rays of elements present in the blood pellets. A 30 mm^2 $\times$ 6 mm Ortec Si (Li) detector

(FWHM = 170 eV at 5.96 KeV) coupled with PC-486 based multichannel analyzer (MCA) was used to collect the fluorescent X-ray spectra of the samples for time durations ranging from 3–5 h. Each spectrum was corrected for the background contribution by channelwise subtraction of the blank cellulose spectrum. Each spectrum was analyzed for photopeak areas corresponding to different elements by using a computer code AXIL (Maunhant & Malmqvist 1992).

The elemental concentrations in various samples were determined using the equation:

$$m_j = \frac{N_{ij}}{i_0 G \varepsilon_{ij} \alpha_{ij} \beta_{ij}},$$

where N_{ij} is the number of counts/s for the ith X-ray photopeak of the jth element, I_0 is the intensity of the photons emitted by the source, G is the geometry factor, α_{ij} is the XRF cross-section of the ith X-rays of the jth element in cm^2/g at the incident photon energy, ϵ_{ij} is the detector efficiency for the ith X-ray of the jth element and β_{ij} is the self absorption correction factor for the target material which accounts for the absorption by the target of the incident photons and emitted characteristic X-rays lying under the ith peak of jth element. The factor $I_0\ G\epsilon_{ij}$ was determined over the energy region 3–16 KeV by collecting K X-ray spectra from thin elemental standard foils of K, Ca, Ti, Sc, V, Mn, Fe, Co, Ni, Zn, Ge, Se, $RbNO_3$, SrF_2, Y and Mo (Micromatter, Deer Harbor, WA, USA) as explained (Dhawan *et al.* 1995; Singh *et al.* 1995) by us earlier.

Serum Li Estimation

Serum Li levels in animals of groups G-III and G-IV were estimated at weekly intervals by EEL flame photometer (Brown & Legg 1970) and were found to be in the physiological range (0.5–1.2 mEq/l of serum) throughout the course of the study.

Results

Typical representative fluorescence X-ray spectra of liver from control and diabetic rats are given in Figures 1 and 2, respectively. X-ray peaks for K, Fe, Cu, Zn, Br and Rb in these spectra are clearly visible. The concentrations of these elements have been calculated by evaluating the area under various X-ray peaks and using the fundamental parameter approach (Gedcke *et al.* 1982; Kumar *et al.* 1989).

The levels of various elements viz., K, Fe, Cu, Zn, Br and Rb in liver samples after dosage period of one month are given in Table 1. The concentrations of different elements are expressed in μg/g dry weight of liver tissues, but for K and Fe, the levels are given in mg/g.

The K contents were found to be significantly lowered in all the treatment groups which was maximum ($p < 0.001$; 28%) in Li treated diabetic rats in comparison with the control group (G-I). Depression in the levels of Rb was noticed in Li treated and Li treated diabetic (G-III and G-IV) groups. However, the levels (Rb) remained unaltered in diabetic (G-II) group. Interestingly, a significant ($p < 0.01$) decline was observed in Fe levels in G-III following Li administration but the levels remained unchanged in G-IV with Li administration to diabetic rats. On the other hand, Li treatment to normal (G-III) and diabetic (G-IV) rats indicated statistically significant ($p < 0.001$) elevation in the levels of Br. A significant elevation ($p < 0.05$) in Cu levels was seen in lithium treated (G-III) group but the levels remained unaltered in lithium treated diabetic rats (G-IV). However, the diabetic (G-II) rats did not indicate any elevation in the Br levels Interestingly, the concentrations of Zn were found to be significantly elevated in all the treatment groups, which was maximum ($p < 0.001$; 37%) in G-III (Li) group. A comparison of various elements from Li treated diabetic group G- IV with the corresponding elements from the diabetic group G-II, implied a significant depression ($p < 0.001$) in K and Rb levels and a significant elevation in the levels of Br.

Discussion

Potassium homeostasis has been documented to be impaired in experimental (Pettit and Vick 1974) and clinical diabetes (Perez *et al.* 1977). Further, a significant inhibition in the hepatic Na^+/K^+ ATP ase activity as documented by Toyoshi and Liu (1980) support our finding of diminished hepatic K contents. They attributed the decline in K contents in diabetic individuals to reduced affinity of Na^+/K^+ ATP ase enzyme system for K, which could be further, ascribed to the insufficiency of insulin, which activates the Na^+/K^+ ATP ase pump.

A significant decline in hepatic K contents following Li treatment in the present study is in conformity with the findings of Lakshmi (1989) who reported a similar reduction in brain astrocytes‘ K contents after Li supplementation in experimental rats. Recently, we reported (Singh *et al.* 1994, 1995) a significant decline

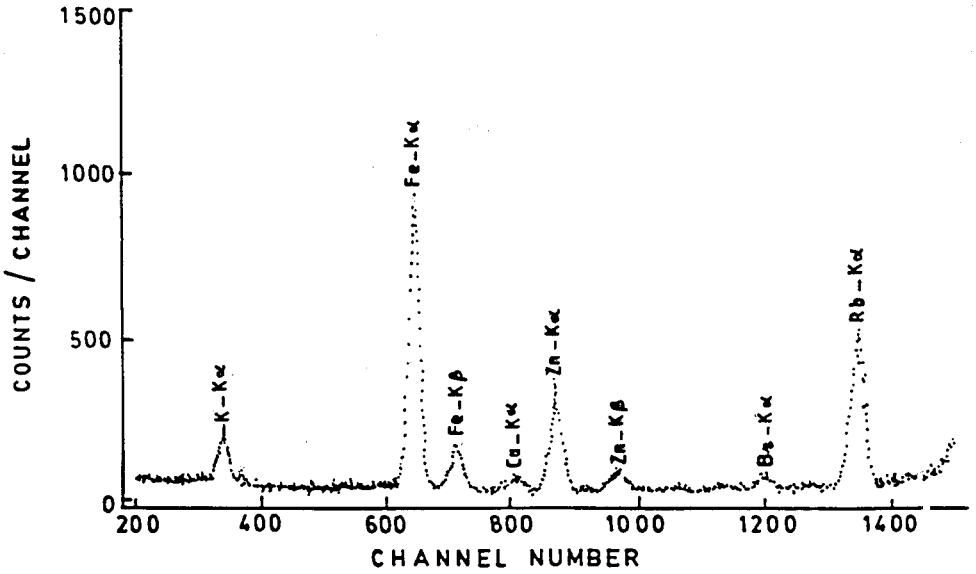

Fig. 1. Typical X-ray spectrum of elements present in control liver sample excited by 17.8 keV photons.

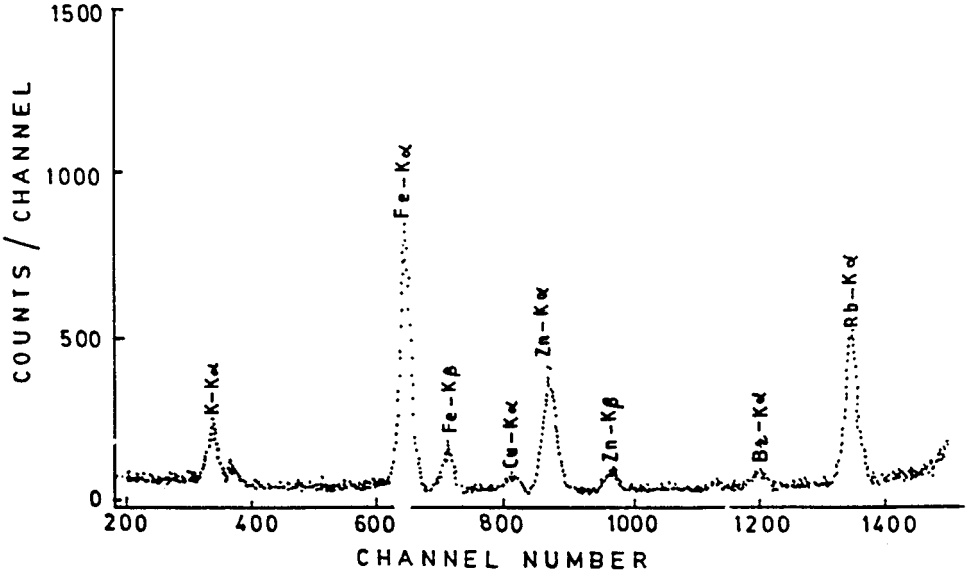

Fig. 2. Typical X-ray spectrum of elements present in diabetic liver sample excited by 17.8 keV photons.

Table 1. Trace elemental profile in control, diabetic, lithium treated and lithium treated + diabetic rats.

Group	Elemental concentrations					
	K	Fe	Cu	Zn	Br	Rb
	(mg/g)		(μg/g)			
Control (G-I)	86.4 ± 8.6	1.13 ± 0.17	22.3 ± 2.6	125.2 ± 9.3	6.8 ± 1.09	69.0 ± 6.4
Diabetic (G-II)	$71.5 \pm 6.1^{**}$	1.07 ± 0.17	25.2 ± 2.4	$144.0 \pm 13.0^{*}$	7.4 ± 1.02	68.1 ± 5.1
Li treated (G-III)	$69.9 \pm 6.0^{**}$	$0.84 \pm 0.14^{**}$	$27.9 \pm 4.1^{*}$	$171.0 \pm 8.5^{***}$	$10.5 \pm 1.1^{***}$	$59.5 \pm 3.2^{**}$
Li treated + diabetic (G-IV)	$61.8 \pm 3.9^{***a1}$	1.1 ± 0.1	5.7 ± 2.6	$152.6 \pm 6.0^{***}$	$9.9 \pm 1.0^{***1}$	$55.0 \pm 3.5^{***2}$

$^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$, when the results are compared with the control group-I.
$^{1}p < 0.01$; $^{2}p < 0.001$ when the results are compared among the lithium treated diabetic group (G-IV), and the diabetic group (G-II).

in blood and brain K contents following 1 and 4 mo. of Li treatment to rats. The observed inhibition in the hepatic Na^+/K^+ ATP ase activity following Li treatment to normal and diabetic rats has also been quoted by Guerri *et al.* (1980). Dick *et al.* (1978) have emphasized that Li alters cellular membrane ATP ase, which is responsible for the regulation of Na^+/K^+ transport across the biomembranes, thus resulting in the altered hepatic K levels. Therefore, the observed depression in K contents in Li treated diabetic animals could possibly be due to cumulative synergistic effect of diabetes and Li treatment to deplete hepatic K stores.

Fe contents remained unaltered in diabetic and Li treated diabetic rats, however, were lowered significantly with Li treatment. In contrast to other essential minerals, excretion of Fe occurs through the erythrocytes destruction. Fe excess is commonly associated with glucose intolerance (Arshag *et al.* 1994). Although the role of Fe in glucose homeostasis appears to be relatively minor (Dandona *et al.* 1983), yet Fe metabolism may be altered in diabetes (Bern & Busick 1985). Arshag *et al.* (1994) reported that foliate deficiency is not a common problem in diabetics but an extensive survey indicated that 3% of individuals had foliate deficiency. A significant reduction in hepatic Fe contents following Li treatment could be assigned to reduction in hemoglobin (Prakash *et al.* 1981) and had also been shown in our earlier studies (Singh *et al.* 1994, 1995).

Zn is an essential constituent of enzymes in many major pathways including the metallo- proteins, thymidine kinase, DNA and RNA polymerase which play important part in protein and nucleic acid metabolism, The role of Zn in carbohydrate metabolism has suggested to mimic insulin and approximately 0.5% of crystal like insulin is Zn (Arshag *et al.* 1994), In the present study, a significant elevation in the hepatic Zn levels was observed in diabetic rats and in accordance with our observation, a rise in Zn levels in streptozotocin and alloxan induced experimental diabetes have been reported (Fallia & Kisser 1981) earlier. Further, hyperzincurea have also been reported in diabetics (Kumar *et al.* 1988; Pidduck *et al.* 1970), in alloxan induced experimental diabetes and depancreatized dogs (Tarui 1963). The observed elevation in hepatic Zn contents may thus be a part of the counter-regulatory mechanism to account for insulin to hepatocytes membranes and suggests the lipogenic effect of insulin in rat adipocytes. The effect of Zn on insulin secretion has been described as biphasic. Some reports (Ghalghazi 1979) indicated that insulin depletion due to pancreatic loss may lead to elevation in Zn levels.

In the present study, a significant elevation in hepatic Zn levels was noticed in Li treated animals. Similar observations were made by Broulik *et al.* (1984), who observed that patients on chronic Li therapy had increased activity of bone isoenzyme of serum alkaline phosphatase for which Zn is a co-factor. Ghoshdastidar (1990) further reported that Li chloride exerts a stimulatory effect on alkaline phosphatase activity in different organs in a dose and time dependent manner. Similarly, Zn levels also got raised in combined Li treated and diabetic rats (G-IV) which is in accordance with the corresponding rise in its levels in individual Li treated and diabetic animals.

Copper is an essential nutrient for all mammals and is a constituent of many enzymes like tyrosine,cytochrome oxidase, ceruloplasmins and other ferroxidases. High concentrations of copper are found in brain, liver and kidney. Although, Cu deficiency is associated with impaired glucose tolerance in experimental animals, yet, it is not problem in diabetics (Cohen *et al.* 1982). Most studies with clinical and experimental diabetes have shown that serum and tissue Cu concentrations are either normal or higher as compared to the normal controls (Walter *et al.* 1989; Noto *et al.* 1983). Similarly, in the present study, we have also found higher (non-significant) hepatic Cu levels in diabetic group which is in agreement with a previous report (Arshag *et al.* 1994), Failla & Kisser (1981) in yet an another study reported elevated Cu contents in diabetic rats and attributed this elevation to raised Cu-metallothionines.

A significant rise was observed in the hepatic Cu contents in the Li treated and Li diabetic groups. Venugopal & Lucky (1978) pointed out that a marked depression in Cu nutrition is brought out by high dietary intakes of calcium carbonate and it was further established that Cu and Ca have synergistic effects (Singh *et al.* 1995). So, the observed rise in hepatic Cu contents may be the consequence of metallothionine induction, as the latter has been reported to be enhanced when glucose metabolism is disturbed.

Bromine (Br), although, a non-essential element, has been detected in all groups. Possibly, the element enters the human system from the environment as had been reported by us (Bandhu *et al.* 1998) earlier. In the present study, hepatic Br contents were found to be raised following Li treatment for 1 mo. Recently, we have reported a similar rise in Br contents in affective disorder patients following Li therapy for up to 12 mo.

Previously, Handrof *et al.* (1989) and Campbell *et al.* (1986) have showed that Br concentrations are higher in-patients receiving Li. These workers considered, this effect was most likely due to Li interfering with the renal excretion of Br. Handrof *et al.* (1989) suggested that the relatively slight increase of Br, in the subtherapeutic range, could have pharmacological effect worthy of further study. In still an another study, Harvey *et al.* (1992) reported that endogenous Br has been found to be raised during Li treatment, and it has been suggested that it may augment the therapeutic effect of Li. They reported that bromine and vanadium concentrations are associated with irregular cortical activity and electroencephalograph (EEG) abnormalities were associated with more side effects of Li. The present finding of raised Br concentrations is also in agreement with our earlier reports (Singh *et al.* 1994, 1995) where a similar rise in blood and brain concentrations of Br following Li treatment for 1 and 4 mo in experimental rats was seen.

On the other hand, no significant change in Br concentrations was seen in diabetic rats and also the levels in Li treated diabetic rats were comparable with the Li treated group.

It was observed that hepatic Rb levels declined significantly in Li and Li treated diabetic rats. Stolk *et al.* (1971) observed that rats treated with rubidium chloride showed an increased shock elevated aggressive behavior. On the contrary, Li have been shown to subside this aggression thereby providing a comparison of the effects of these two salts on a common ailment. Further, since Rb and Li are positioned in the periodic table in the same group (1A), they may have synergistic effects (Singh *et al.* 1994). However, diabetes seems to have no effect on the Rb metabolism as evidenced by non-significant changes in Rb levels in diabetic and Li treated diabetic groups w.r.t the only Li treated group.

References

Amdisen A. 1988 Clinical features and management of lithium poisoning. *Med Toxicol* **3**, 18–32.

Arquilla ER, Packer S, Tamas W, Miyamoto S. 1978 The effect of Zn on insulin metabolism. *Endocrinology* **103**, 1440–1449.

Arshag DM, Mask F, Byron H, Melinda M, Judith WR. 1994. Selected vitamins and minerals in diabetes, *Diabetes Care* **17**: 464–479.

Bandhu HK, Puri S, Shahi JS, Mehta D, Garg ML, Singh N, Mangal PC, Suri CR, Swietlicki, Trehan PN. 1996. An evaluation of the sources of air pollution in the city of chandigarh, India: A study using EDXRF technique. *Nucl Instr Meth Phys Res B*. **114**, 341–344.

Bern MM, Busick EJ. 1985 Disorders of blood and diabetes. In Marble A, Krall LP, Bardley RF, Chhristlieb AR, Soelder JS, eds. *Tolins diabetes* Mellitus. Philadelphia. PA Lea and Febiger, 748–752.

Birch, NJ, Jenner FA. 1973. The distribution of lithium and its effects on the distribution and excretion of other ions in the rat. *Br J Pharmacol* **47**, 586–589.

Broulik PD, Stepan JJ, Soucek K, Pacovsky V. 1984 Alteration in human serum alkaline phosphatase and its bone isoenzyme by chronic administration of lithium. *Clinic Chim Acta* **140**, 151.

Brown PB, Legg EI. 1970 The estimation of lithium in serum. *Ann Clin Biochem* **7**, 13.

Campbell CA, Peet M, Ward NJ. 1989 Trace elemental serum levels in lithium-treated patients. In: Birch NJ, ed. *Lithium: Inorganic Pharmacology and Psychiatric Use*. Oxford: IRL Press; 309–310.

Cohen AM, Teitelbaum A, Miller E, Ben Tir Y, Hist R, Field M. 1982. The effect of copper and carbohydrate metabolism. *Isr J Med Sci* **18**, 840–844.

Dandona P, Hussain MA, Varghes Z, Palitis D, Flymn DM, Hoffbrand AR. 1983. Insulin resistance and iron overload. *Ann Clin Biochem* **20**, 77–79.

Dhawan D, Singh B, Chand B, Singh N, Mangal PC, Trehan PN. 1995 X-ray flurescence in the assessment of inter elemental interactions in rat liver following lead treatment. *Biometals* **8**, 105–110.

Dick DAT, Naylor M, Dick EG. 1978 In Johnson FH and Johnson SP eds. *Lithium in Medical Practice* Lancaster: MTP Press; 173.

El-Mallakh RS. 1986 Acute lithium neurotoxicity. *Psychiatr Dev* **4**, 311–328.

Failla ML, Kiser RA. 1983 Hepatic and renal metabolism of copper and zinc in the diabetic rat. *Am J Physiol* **244**, E113–E121.

Gadallah MF , Feinstein EI, Massry SG. 1988 Lithium intoxication: Clinical course and therapeutic considerations. *Miner Electrolytic Metab* **14**, 146–149.

Gedcke DA, Byars L, Jacobus N. 1982 FPT- an integrated fundamental parameter for broad band EDXRF analysis without a set of similar standards. *Adv X-ray Anal* **26**, 355.

Gershon S. 1970 Lithium in mania. *Clin Pharmacol Ther* **11**, 168.

Ghalghazi T, Ludvigsen CW, Mc Daniel ML, Lacy PE. 1979 The inihibitory effect of zinc insulin secretion. *IRCS J Med Sci* **7**, 12.

Ghosdastidar D. 1990 Long-term effect of lithium on brain regional catecholamne metabolism. *Ind J Exp Biol* **28**, 444–449.

Guerri C, Ribelles M, Grisalia S. 1980 Effect of lithium and alcohol administration on Na^+/K^+ ATPase. *Biochem Pharmacol* **30**, 25.

Handrof CR, Coleman JH, Rawls WN. 1989 Elevated serum bromine in patients taking lithium carbonate. *J Clin Psychiatr* **46**, 9–10.

Hare JU, Judith S, Stern, Gerald M, Reaven, Keen CL. 1985 The effect of maternal diabetes on trace element status and fetal development in the rat. *Diabetes* **34**, 1031–1040.

Harvey NS, Jarratt J, Ward NI. 1992 Trace elements and the electroencephalogram during long term lithium treatment. *Br J Psychiatr* **160**, 654–658.

Haugaard ES, Mickel RA, Haugaard N. 1974. Actions of lithium ions and insulin on glucose utilisation, glycogen synthesis and glycogen synthase in the isolated rat diaphragm. *Biochem Pharmacol* **23**, 1675.

Jefferson JW, Griest JW. 1987 Lithium:a practitioner's guide. *Hosp Ther* **12**, 87–89.

Kumar S, Rao KSJ. 1974. Blood and urinary zinc levels in diabetes mellitus. *Nutr Metab* **17**, 231.

Kumar S, Singh S, Mehta D, Garg ML, Mangal PC, Trehan PN. 1989 Matrix corrections for quantitative determination of trace

elements in biological samples using energy dispersive X-ray fluorescence spectrometry. *X-ray Spectrometry* **18**, 207.

Lakshmi GYCVS. 1989 Effect of lithium on regulation of two molecular forms of Na^+/K^+ ATPase in rat brain. *Ind J Exp Biol* **27**, 903.

Lieff S, Herrmann N. 1989 Lithium treatment of a patient with Addison's disease and affective psychosis. *Pharmapsychiatr* **22**, 84–86.

Maunhaut W, Malmqvist KG 1992 Particle induced X-ray emission analysis. In Van Gricken RE, Markowicz AA, eds. *Handbook of X-ray Spectrometry*. New York: Marcel Dekker, 517–581.

Mendels J, Secud SK, Landerback DW. 1981 A controlled study of antidepressant effects of lithium carbonate. *Arch Gen Psychiatry* **26**, 154.

Monking HS. 1989 Lithium treatment of a patient with Addison,s disease and affective psychosis. *Phamacopsychiatr* **22**, 84–86.

Noto R, Alicata R, Sfogliano L, eris S, Bifarella M. 1983 A study of cupremia in a group of elderly diabetes. *Acta Diabetol* **20**, 81–85.

Nyfeler F, Fasel P, Walter P. 1981 Short term stimulation of net glycogen production by insulin in rat hepatocytes. *Biochem Biophys Acta* **675**, 17.

Pai M, White AC, Deane AG. 1986 Lithium augmentation in the treatment of delusional depression. *Br J Psychiatr* **148**, 736–738.

Perez GO, Hespier L, Knowles R, Oster JR, Voamonde CA. Contribution of pancreatic insulin to extra renal potassium homeostasis: a tow compartmental model. 1977 *Arch Intern Med* **137**, 1018.

Pettit GW and Vick RL. 1974 Potassium homeostasis in chronic diabetes mellitus. *Am J Physiol* **226**, 319.

Pidduk HG, Wren PJJ, Evans DAP. 1970. Hyperzincuria of diabetes mellitus and possible genetical implications of this observation. *Diabetes* **19**, 240.

Plenge P, Mellerup ET, Rafaeson OT. 1970 Lithium action on glycogen synthesis in rat brain, liver and diaphragm. *J Psychiatr Res* **8**, 29–33.

Prakash R, Sethi N, Agrawal SS, Kushwaha MR, Sethi BB. 1981 A case report of megaloblastic amemia secondary to lithium. *Am J Psychiatr* **38**, 849.

Rao NV, Reddy SB, Narayana DGS, Satyanarayana g, Sastry DL. 1988 Quantitative estimates of Patassium in whole blood samples from Diabetics. *X-ray Spectrometry* **17**, 33–35.

Rossetti L. 1989. Normalisation of insulin sensitivity with lithium in diabetic rats *Diabetes* **36**, 648–652.

Schou M. 1976 Lithium in Psychiatric therapy and prophylaxis. *Annu Rev Pharmacol Toxicol* **16**, 231.

Schou M. 1986 Lithium treatment: a refresher course. *Br J Psychiatr* **1491**, 41.

Schrauzer GN *et al.* 1992 Lithium in scalp hair of adults, students and violent criminals. Effect of supplementation and evidence for interactions with B12 and with other trace elements. *Biol Trace Element Res* **34**, 161.

Singh B, Dhawan D, Mangal PC, Chand B, Singh N, Trehan PN. 1994 Combined action of lead and lithium on essential and non essential elements in rats blood. *Biol Trace Elem Res* **46**, 79–82.

Singh B , Chand B , Dhawan D , Mangal PC, Trehan PN. 1995 Trace element distribution in rat brain following lead and lithium supplementation – A study using an EDXRF spectrometer. *Appl Radia Isot* **46**, 59–64.

Singh B, Bandhu HK, Pathak CM, Garg ML, Mittal BR, Kulhara P, Singh N, Deodhar SD. 1998 Effect of lithium therapy on trace elements in blood of psychiatric patients. *Trace Elem Electrolytes* **15**, 94–100.

Stolk JM, Corner RL, Berchange JD. 1971 Rubidium-induced increase in shock-elicited agression in rats. *Psychopharmacology* **22**, 250–255.

Toyoshi O, Liu M. 1980. Effects of alloxan diabetes on the sodium potassium adenosine triphosphate enzyme system in dog hearts. **962**, 799.

Venugopal B, Lucky, TD. 1978 *Metal Toxicity in Mammals*, 2nd ed. New York: Plenum Press.

Vestergaard P , Poulstrup I, Schou M. 1988 Protective studies on a lithium cohort, Tremor, weight gain, diarrhea, psychplogical complaints. *Acta Psychiatr Scand* **78**, 434–441.

Walter RM, Uriu-Hare JY, Olin KL, Oster MH, Anawalt BD, Critchfield JW, Keen CL. 1981 Copper, zinc, manganese, magnesium status and complications of diabetes mellitus. *Diabetes Care* **14**, 1050–1056.

Watanabe S, Taguchi K, Nakaya K, Noma T, Yokoyama S, Otsuki S.1973 Lithium carbonate treatment in manic psychosis. *Fotea Psychiatr Neurol Jpn* **27**, 183–196.