34 Toole, B.P., Glycosaminoglycans in morphogenesis, in: Cell Biology of Extracellular Matrix, p.259–294. Ed. E.D. Hay. Plenum Press, New York 1981.
35 Vallee, B.L., Riordan, J.F., Lobb, R.R., Higashi, N., Fett, J.W., Crossley, G., Buhler, R., Budzik, G., Breddane, K., Bethune, J.L., and Alderman, E.M., Tumor-derived angiogenesis factors from rat Walker 256 carcinoma: an experimental investigation and review. Experientia *41* (1985) 1–15.
36 Wohlwend, A., Montesano, R., Vassalli, J.-D., and Orci, L., LLC-PK_1 cysts: a model for the study of epithelial polarity. J. cell. Physiol. *125* (1985) 533–539.
37 Yamada, K.M., Akiyama, S.K., Hasegawa, T., Hasegawa, E., Humphries, M.J., Kennedy, D.W., Nagata, K., Urushihara, H., Olden, K., and Chen, W.T., Recent advances in research on fibronectin and other cell attachment proteins. J. Cell Biochem. *28* (1985) 79–97.
38 Yang, J., and Nandi, S., Growth of cultured cells using collagen as substrate. Int. Rev. Cytol. *81* (1983) 249–286.
39 Yang, J., Richards, J. Bowman, P., Guzman, R., Enami, J., McKormick, K., Hamamoto, S., Pitelka, D., and Nandi, S., Sustained growth and three-dimensional organization of primary mammary tumor epithelial cells embedded in collagen gels. Proc. natn. Acad. Sci. USA *76* (1979) 3401–3405.

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Stable isotopes of lithium: dissimilar biochemical and behavioral effects

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Summary. Lithium, which is used routinely in the treatment of mania, is composed of two stable isotopes, lithium-7 (92.58%) and lithium-6 (7.42%). Usually there is minimal physiological or biochemical differentiation between isotopes of an element, but lithium is an exception. Data derived from a variety of biochemical and behavioral experiments are reviewed to support this idea. Additionally, the clinical implications of this work are presented.
Key words. Lithium; isotopes; lithium-6; lithium-7; mania.

Use of isotopes of an element has been invaluable in studies of a variety of problems including metabolic pathways, enzyme reaction mechanisms and the distribution of administered drugs. Isotopes of lighter elements have significant mass differences, which could be manifested by unexpected differences in biological systems. However, when isotopes are incorporated into comparatively large molecules, differences between their masses (i.e. hydrogen, deuterium and tritium) are reduced because of high overall molecular weights. In the case of lithium (Li) it is the ion, Li^+, that is biologically active.

As a consequence of its small mass, Li^+ possesses physicochemical properties different from the other alkali metal ions, sodium (Na^+), potassium (K^+), rubidium (Rb^+) and cesium (Cs^+), with respect to solubility, formation of complexes and magnitude of the radius of hydration. Li exhibits properties intermediate between the alkali and alkaline earth elements; it is similar to magnesium (Mg) with respect to ionic radius and calcium (Ca) with respect to charge density. The uniqueness of Li^+ is primarily a result of the small size of its ionic radius and a capability of polarization superior to the other alkali metal ions, which leads to solvation and covalent bond formation[7]. As would be expected Li^+ has both the biggest charge/radius ratio and radius of hydration of the alkali metal ions; these combined effects have a profound influence on the transport of Li^+ ions across the cell membrane.

Naturally occurring lithium (Li-N) is composed of two stable isotopes: lithium-7 (Li-7) is the major (92.6%) and lithium-6 (Li-6) is the minor constituent (7.4%). Nuclei of Li-6 contain three protons and three neutrons, Li-7 three protons and four neutrons. Radii of hydration of $(Li\text{-}6)^+$ and $(Li\text{-}7)^+$ are not the same because of differences in the charge/mass ratios of the unhydrated ions. The associated changes in the electrostatic interaction between the isotope ions, water molecules and negatively charged membrane species could contribute to dissimilarities in transport of the two Li isotopes across membranes. Isotopically pure Li-6 and Li-7 salts are commercially available.

Previous references to the utilization of Li-6 in biological systems are not abundant. Thellier et al.[21] used Li-6 to determine the distribution patterns of Li in the rat with a technique related to conventional autoradiography. Li-6 was also used as a tracer for Li-7 in a pharmacokinetic study by Birch et al.[4]. A limited number of reports is found in the literature dealing with the chronic toxicology of the stable Li isotopes and their possibly different effects. Rats maintained on small quantities of Li-6 showed no obvious signs of toxicity and four human subjects received Li-6 without experiencing any ill effects[4,5]. However, recent studies have demonstrated differential effects of the Li isotopes in a variety of systems using biochemical, pharmacological, behavioral and toxicological approaches. An internal consistency is apparent with respect to the data obtained in these studies, regardless of the specific methodology used and an isotope effect is clearly indicated.

Until recently it has been tacitly assumed that toxicity of the two Li isotopes was equivalent primarily because isotopically pure Li-6 administered for a year in drinking

water produced no obvious effects in rats[5]. However, when massive amount of the two Li isotopes were administered i.p. in equal doses in mice, Li-6 proved to be more lethal than Li-7[1]. Li-N had a lethality that was intermediate between Li-6 and Li-7. When the toxicity data was expressed in terms of 50% lethal dose (LD-50), the LD-50's for Li-6, Li-N and Li-7 were 13 meq/kg, 15 meq/kg and 16 meq/kg, respectively. Mice given Li-6 became lethargic 5–10 min after administration; mice treated with Li-7 appeared to be more alert.

Four groups of Swiss-Webster mice were given drinking fluid containing the chlorides of Li-6, Li-7 and Li-N or water for six weeks[2]. Li taste was masked with saccharin. The Li-6 group displayed greater changes than the Li-7 group. All Li-treated mice showed less gain in weight than controls, but only Li-6 mice showed weight loss. Li-6 mice experienced a greater decrease in spontaneous motility. Mortality was higher in Li-6 than Li-7 animals (80% versus 20%) and deaths occurred earlier than in Li-6 animals.

Spontaneous motor activity in experimental animals is known to be decreased after treatment with Li. Rats given Li-N for up to ten days showed a significant decrease in exploratory behavior and motility[9,10,15,19]. Isotopically pure Li-6 and Li-7 (1.5 meq/kg twice daily) administered i.p. to rats decreased motility; Li-6 initially produced a more profound effect than Li-7[11]. The differential behavioral effect of the isotopes proved to be time dependent, being most evident on the third day of treatment and disappearing by the fifth treatment day. On the third day of treatment the mean motility of the Li-6 group was 52% of the pre-Li value ($p < 0.02$); the mean motility of the Li-7 group was 82% of the pre-Li value ($p < 0.05$). Of interest was the difference in motility on the third treatment day between ranked pairs of Li-6 and Li-7 animals, which was statistically significant at a confidence level of $p < 0.05$.

Pharmacokinetic studies were performed in vivo in cats to determine the distribution of the Li isotopes between plasma and CSF. Adult cats were given a single dose acutely of either Li-6 or Li-7 in the chloride form by intubation (1 meq/kg)[20]. The mean plasma level of Li-6 was consistently higher than Li-7 between two and nine hours post-administration. The elimination curve for the isotopes was biphasic; the slow component for Li-6 had a mean half-life of 12.9 ± 0.69 (SE) h and for Li-7, 15.9 ± 1.28 (SE) h ($p < 0.01$). The determinations of simultaneous CSF and plasma kinetics provided a convincing demonstration of the differential handling of Li-6 and Li-7. The plasma and CSF concentrations of Li-6 were higher than those of Li-7. The CSF/plasma ratio for Li-6 was greater than Li-7 ($p < 0.05$), indicating a differential distribution of the two isotopes at the blood-brain barrier.

In studies of the effects of Li in pregnancy and early development in rats, chlorides of Li-6, Li-7 or Li-N were given orally to 3-month-old females prior to and during gestation and lactation[17]. Two dosage schedules were used, 2 meq/kg and 4 meq/kg. At the low dose alterations in maternal behavior and delays in early development were found. Effects were more pronounced at the higher dose, primarily in the development of visual depth perception. Li-6 at both doses appeared to have a stronger effect on maternal behavior and development than Li-7. Maternal effects were transient, gradually disappearing after cessation of Li ingestion; effects in offspring persisted for at least four months after treatment was terminated.

With an in vitro uptake technique it was demonstrated that the human erythrocyte membrane differentiated Li-6 from Li-7[12]. The quantity of Li-6 which accumulated in the erythrocyte was found to be greater than Li-7 with the ratio of concentrations of Li-6/Li-7 in the erythrocyte ranging from 1.05–1.08. On the basis of the uptake data an isotope effect of 5.4–8.5% appeared to exist between Li-6 and Li-7. More Li-6 was taken up by the erythrocytes than Li-7 at the end of three and four hours of incubation in homologous plasma from either males or females. The difference was statistically significant (males: 3 h, $p < 0.025$; 4 h, $p < 0.01$; females: 3 h, $p < 0.025$; 4 h, $p < 0.05$) using paired comparison t tests. The combined data for erythrocytes from both males and females also indicated a statistically significant larger accumulation for Li-6 than Li-7 for both incubation times (3 h, $p < 0.01$; 4 h, $p < 0.05$) using paired comparison t tests.

Li salts are routinely administered in the treatment of manic-depressive illness[3]. Taken for long periods of time these salts may produce toxic side effects, including kidney damage[3]. Intake during pregnancy, while discouraged, does occur and raises questions of possible deleterious effects on offspring development[16,23]. Of course salts used in therapy contain both Li-6 and Li-7. No data on therapeutic effectiveness of isotopically pure preparations are available. However, it has been established at the membrane level that manic patients given Li-N chloride were able to distinguish Li-6 from Li-7[14]. Thirty manic patients 21–77 years of age treated with Li from 4 to 156 months participated in the study. Using a modification of the method of Brost et al.[6] the isotopic abundances in erythrocytes and plasma were determined by atomic absorption spectrophotometry. Had no isotopic fractionation occurred, the ratio of Li-6 abundances in erythrocytes and plasma would have been unity. The ratio of abundances was 1.271 ± 0.041 (SE) with a significance of $p < 0.01$, demonstrating that the erythrocyte membrane had the in vivo capability of distinguishing Li-6 from Li-7. The percentage of Li-6 in the patient erythrocytes was enhanced by a factor greater than 25%. Sherman et al.[18] corroborated the differential treatment of Li-6 and Li-7 by a biological membrane. They found that the rat cerebral cortex was able to distinguish between the isotopes of Li. After a s.c. administration of equal doses of the two isotopes the ratio of Li-6/Li-7 in the cerebral cortex was 1.5. Furthermore, Li-6 enhanced myoinositol-1-phosphate activity more than Li-7, 26.8% versus 17.7%, respectively. It was concluded that the differential effects of Li-6 and Li-7 on inositol metabolism can be explained by the ability of brain tissue to take up the two isotopes differentially.

A possible unifying concept in all these studies may be the relationship between the observed effects and Li^+ ions crossing a membrane or series of membranes. Accumulated Li-N transport data indicate that a Li^+ ion crossing a membrane requires energy-dependent and passive diffusion mechanisms[15]. An assumption is made that iso-

topes of Li are initially transported in the hydrated form. Based upon lattice constant measurements the ionic volume of $(Li\text{-}6)^+$ is greater than $(Li\text{-}7)^+$[8,22]. However, it is the hydrated form $(Li\text{-}7)^+(H_2O)$ that has a greater ionic volume than $(Li\text{-}6)^+(H_2O)$. Because of its smaller ionic volume, the passage of $(Li\text{-}6)^+(H_2O)$ by passive diffusion through a membrane channel would be more rapid than the passage of $(Li\text{-}7)^+(H_2O)$. An energy dependent mechanism of transport would of necessity make an explanation more complex.

Because of the differential treatment of the two Li isotopes by membrane systems in vitro and in vivo, the possibility was raised that harmful sideeffects of Li during prolonged clinical use in manic-depressive patients might be reduced by administering only Li-7, the less toxic of the two stable isotopic forms. There is ample justification for clinical trials of this hypothesis because of the data indicating both the greater in vivo toxicity and the more rapid penetration in vitro of Li-6 than Li-7. Whether Li-7 alone would have therapeutic efficacy equivalent to Li-N remains to be established.

The finding that biological systems can discriminate between isotopes of a given element is in itself provocative. Relative rates at which isotopes cross biological membranes might be used to provide further information regarding the mechanisms of ion transfer. They could also provide further information pertinent to explaining the mechanisms of the differential effects of these isotopes on neuronal activity and behavior. Finally, these observations may have clinical relevance for ameliorating toxicity in clinical disorders for which Li treatment is indicated.

1 Alexander, G., Lieberman, K.W., and Stokes, P., Differential lethality of Li isotopes in mice. Biol. Psychiat. *15* (1980) 469–471.
2 Alexander, G., Lieberman, K.W., Okamoto, M., and Stokes, P., Stable isotopes of Li: effects on behavior in rodents. Fedn Proc. *41* (1983) 1583.
3 American Psychiatric Association Taskforce, The current status of Li therapy. Am. J. Psychiat. *132* (1975) 997–1007.
4 Birch, N.J., Robinson, D., Ince, R.A., and Hullin, R.P., Li-6 stable isotope determination by atomic absorption spectrophotometry and its application to pharmacokinetic studies in man. J. Pharm. Pharmac. *30* (1978) 683–685.
5 Birch, N.J., in: Lithium in Medical Practice, pp. 87–144. Eds F.N. Johnson and S. Johnson. University Park Press, Baltimore 1978.
6 Brost, D.F., Brackett, J.M., and Bunch, R.W., Determination of Li by optically monitored stable isotope dilution. Analyt. Chem. *51* (1979) 1512–1516.
7 Cotton, F.A., and Wilkinson, G., Advanced Inorganic Chemistry, 3rd Edn. Interscience, New York 1975.
8 Covington, E.J., and Montgomery, D.J., Lattice constants of separated Li isotopes. J. Chem. Phys. *27* (1957) 1030–1032.
9 Greenspan, K., Aronoff, M., and Bogdanski, D., Effects of lithium carbonate on turnover and metabolism of norepinephrine in rat brain. Pharmacology *3* (1970) 129–136.
10 Johnson, F.N., and Wormington, S., Effect of Li on rearing activity in rats. Nature, New Biol. *235* (1972) 159–160.
11 Lieberman, K.W., Alexander, G., and Stokes, P., Dissimilar effects of Li isotopes on motility in rats. Pharmac. Biochem. Behav. *10* (1979) 933–935.
12 Lieberman, K.W., Stokes, P., and Kocsis, J., Characteristics of the uptake of Li isotopes into erythrocytes. Biol. Psychiat. *14* (1979) 845–849.
13 Lieberman, K.W., Meltzer, H.L., and Stokes, P., Transport studies of Li isotopes. Fedn Proc. *39* (1980) 405.
14 Lieberman, K.W., Chen, C., Mann, J., and Rubino, R., Erythrocyte differentition of naturally occurring isotopic Li abundances. Pharmac. Biochem. Behav. *23* (1985) 145–146.
15 Perez-Cruet, J., Tagliamonte, A., Tagliamonte, P., and Gessa, G.L., Li and motility studies in rats. J. Pharmac. expl Ther. *178* (1971) 325–330.
16 Schou, M., What happened to Li babies: a followup study of children without malformations. Acta psychiat. scand. *54* (1976) 193–197.
17 Sechzer, J., Lieberman, K.W., Stokes, P., and Falasco, J., Effects of isotopically Li on maternal behavior, development and learning. Pharmacologist *24* (1982) 196.
18 Sherman, W.R., Munsell, L.Y., and Wong, Y., Differential uptake of Li isotopes by rat cerebral cortex and its effect on inositol phosphate metabolism. J. Neurochem. *42* (1984) 880–882.
19 Smith, D.F., and Smith, H., Effect of prolonged Li administration on activity, reactivity and endurance in the rat. Psychopharm. *30* (1973) 83–88.
20 Stokes, P., Lieberman, K.W., Okamoto, M., and Alexander, G., Stable isotopes of Li: in vivo differential distribution between plasma and CSF. Biol. Psychiat. *17* (1982) 413–421.
21 Thellier, M., Stelz, T., and Wissocq, J., Detection of stable isotopes of lithium or boron with help of a (n, alpha) nuclear reaction. Biochim. biophys. Acta *437* (1974) 604–627.
22 Thewlis, J., Unit-cell dimensions of LiF made from Li-6 and Li-7. Acta crystallog. *8* (1955) 36–38.
23 Weinstein, M.R., The international register of Li babies. Drug Inf. J. *10* (1976) 94–100.

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Full Papers

Long-term motor activity recording of dogs and the effect of sleep deprivation

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Summary. Motor activity of laboratory dogs was recorded for several weeks with an ambulatory monitoring device. The effect of 24 h sleep deprivation (SD) on motor activity during recovery was investigated. A clear rest-activity rhythm was established. The dogs exhibited a similar mean daily rest-activity pattern: 1) rest occurred mainly in the dark; 2) the amimals were most active after light onset; activity increased during the last two dark hours; 3) a rest period was found at noon and reduced activity during afternoon hours. There was a marked difference in total activity