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EFFECTS OF LITHIUM ON CEREBRAL RNA METABOLISM *IN VITRO* AND *IN VIVO*

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Abstract—The effect of Li^+ on cerebral RNA metabolism has been studied in rats. Li^+ could not replace Mg^{2+} as an essential co-factor for RNA polymerase but stimulated the enzyme in the presence of optimum concentrations of Mg^{2+}. The stimulation was less than that achieved with Na^+ and K^+ and differed from these in being biphasic with respect to concentration. A similar biphasic effect of Li^+ was seen with liver RNA polymerase and brain Poly C synthetase. The action of Li^+ on pancreatic and brain ribonuclease resembled that of Na^+. Li^+ did not reduce the inhibitory effect of Mg^{2+}.

Chronic LiCl treatment in rats (0·15 mg/g/day for 13 days) did not significantly alter the rate of RNA synthesis, the RNA content nor RNA composition in the brain. It is concluded that the changes in uric acid excretion in human manic depressives during lithium-induced remission are not reflections of a direct or indirect effect of Li^+ on cerebral RNA metabolism.

THE MECHANISMS involved in manic depressive illness are, as yet, unknown but this disease and the alleviation of its symptoms by treatment with lithium has been the subject of a great deal of research. Lange[1] first pointed out the beneficial effects of lithium in both gout and mental depression and, in modern times, lithium therapy is playing an increasing role in the management of chronic manic conditions and manic depressive illness.[2,3] However the mode of action of lithium has remained uncertain, there being two main schools of thought: that favouring an effect on sodium transport and electrolyte balance[4] and that favouring an effect on catecholamines, in particular serotonin.[5] However some workers[6] have found that chronic administration of lithium had no effect on brain neuroamines. A multiplicity of other effects of lithium have been reports e.g. on brain glutamic acid,[7] carbohydrate metabolism,[8] endocrine function[9] and on certain circadian rhythms.[10]

Anumonye *et al.*[11] showed that changes in mood in manic depression were closely followed by changes in daily uric acid excretion. Excretion was increased markedly in the early phase of natural or lithium induced remission from both hypomanic and depressive episodes. The plasma uric acid level remained constant. While this could be simply a uricosuric effect it is possible that since uric acid is the major end product of purine catabolism, these fluctuations could be reflecting alterations in cerebral RNA metabolism. It is known that the RNA metabolism in the brain is affected by nervous activity[12–14] and factors such as stress can markedly alter RNA turnover.[15] Chronic lithium treatment has been shown to reduce the content of cytoplasmic RNA in certain cells[17] and at concentrations of 0·2 M to dissociate ribosomes in *E. coli*.[18]

Lithium, a Group 1 alkali metal, shows the typical properties of a first row element in the periodic table in that it has strong diagonal affinities with the next group. Thus

lithium, in addition to resembling sodium and potassium, shows marked similarities to magnesium, e.g. in having a carbonate and phosphate of low solubility. Since sodium, potassium and magnesium all have effects on the activity of the enzymes involved in RNA metabolism,[19] we have made a number of studies both *in vitro* and *in vivo* to determine whether lithium had any direct or indirect effects on cerebral RNA metabolism. Part of his work has been published in the form of a preliminary communication.[20]

MATERIALS AND METHODS

Estimation of RNA polymerase. Brains from fasted 150–200 g male albino Wistar rats were removed, washed with ice cold saline and homogenized in a homogenizer of pestle clearance 10 thou. The homogenizing medium was 0·32 M sucrose, 1 mM-$MgCl_2$ and 1 mM-K-phosphate (pH 6·5). 15 ml was used per gram of tissue. The homogenate was centrifuged at 850 *g* for 10 min. This and all subsequent operations were performed at 0–4°. The crude nuclear pellet was washed twice with homogenizing medium and recentrifuged at 850 *g* for 10 min. The partially purified pellet was resuspended in 20 ml (per gram original wet weight) of 2·0 M-sucrose, 1 mM-$MgCl_2$ and 1 mM-K-phosphate, pH 6·5 and centrifuged for 45 min at 63,000 *g*. The purified nuclear pellet had a RNA/DNA ratio of 0·23 ± 0·02 and a protein/DNA ratio of 2·87 ± 0·37. These ratios provide a useful index of purity. The present values compare favourably with those obtained by other investigators.[21,22] In some experiments the pellet was suspended in 2·2 M sucrose, 1 mM-$MgCl_2$ and 1 mM-K-phosphate pH 6·5 and layered over an equal volume of 2·47 M-sucrose in the same buffer. Recentrifuging at 64,000 *g* for 45 min resulted in two nuclear fractions: one enriched in neuronal nuclei and the other enriched in glial nuclei. This method for separating brain nuclei was based on that of Burdman.[23]

Nuclear pellets were suspended in 0·25 M-sucrose + 1 mM-$MgCl_2$ to a final DNA concentration of 50 μg/0·1 ml. The RNA polymerase activity was assayed by incubation in the medium of Widnell and Tata.[24] This contained in 0·5 ml: 50 μmole Tris–HCl buffer, pH 8·1, 2·5 μmole $MgCl_2$, 35 μmole KCl, 10 μmole Cysteine, 3 μmole NaF, 0·3 μmole each of GTP, CTP and UTP, 0·015 μmole non-radioactive ATP, 0·005 μmole [U-^{14}C]ATP (sp. act. 196 mCi/mmole) 0·1 ml nuclear suspension. All incubations were performed at 37° and were commenced by the addition of the nuclear suspension. Preliminary experiments indicated that the incorporation of labelled ATP into RNA was linear for 15 min. Consequently all incubation were carried out for this period. Incubations were terminated by the addition of 5 ml 0·5 N-$HClO_4$ at 0° followed by 1 ml of a 2 mg/ml solution of yeast RNA to act as carrier. Controls were provided by terminating duplicate incubations at time zero. The precipitate was washed twice with 4 ml 0·2 N-$HClO_4$, twice with 3:1 (v/v) ethanol–diethyl ether (these washes at 0–4°) and then digested for 18 hr with 1 ml 0·3 N-NaOH at 37°. 0·1 ml 50% TCA was then added at 0° and after centrifugation the radioactivity of the supernatant was determined by scintillation counting. The DNA content of the precipitate was estimated by the diphenylamine method.[25]

Although the bulk of the present work used the method described above since the use of intact nuclei was preferable on physiological grounds, some work was also performed as the 'aggregate enzyme' preparation of Barondes.[26] Nuclear pellets were suspended in 0·05 M-Tris–HCl buffer, pH 7·5 (to the same volume as the original brain

homogenate) and homogenized with 10 strokes of a pestle with clearance 3 thou. The suspension was centrifuged at 12,000 *g* for 10 min after being chilled for 5 min on ice. The resulting pellet was suspended in half its volume of the pH 7·5 buffer and 2 M-KCl added dropwise to a final concentration of 0·5 M. The gelatinous precipitate was lifted out on a glass rod, washed three times with 2 ml of the pH 7·5 buffer, and rehomogenized with 10 strokes of the pestle (clearance 3 thou). The suspension was centrifuged at 1000 *g* for 10 min and the resulting pellet suspended in 0·05 M-Tris–HCl buffer (pH 8·0) to a final concentration of 10 mg protein/ml. This enzyme preparation was capable of incorporating [U-^{14}C]GTP (sp. act. 575 mCi/mmole, Radiochemical Centre, Amersham) into RNA for 2 hr if a phosphoenolpyruvate–pyruvate kinase energy generating system was present but in the bulk of the present studies the simple medium of Weiss[27] was used. This gave linear incorporation of [^{14}C]GTP for 20–30 min. Incubations were terminated and the RNA extracted by the method described for the intact nuclei.

Estimation of poly C synthetase activity. This enzyme was of interest since it is believed to be restricted to neuronal nuclei[28] and because homopolymerases may have a role in the regulation of RNA synthesis in the nucleus.[29] The method of assay was similar to that used for estimating RNA polymerase activity in intact nuclei except that all UTP, GTP and ATP were omitted from the incubation medium and replaced by 0·02 μmole of [U-^{14}C]CTP (sp. act. 433 mCi/mmole).

Estimation of ribonuclease activity. The assay of ribonuclease is complicated by the fact that this enzyme is sensitive to changes in ionic strength and that these changes can alter the pH optimum.[30] The situation is complicated further by a number of different ribonucleases present in the brain. However, the object of the present study was simply to determine whether Li^+ resembled or differed from Na^+ and Mg^{2+} in its effect on ribonuclease or antagonized the effects of either of these ions.

Initial experiments were performed on pancreatic ribonuclease using a spectrophotometric method.[31] To study the effects of the single cations 1 mg yeast RNA was incubated with 0·05 mg pancreatic ribonuclease (40–50 Kunitz units/mg) in 3 ml distilled water. The concentrations of NaCl, LiCl and $MgCl_2$ were varied from 0 to 400 mM. The reaction mixture was incubated at 37° in a Silica cuvette and the extinction at 300 nm measured at half minute intervals, until a constant final value was attained. Under this condition the breakdown of RNA results in an exponential decrease in the extinction at 300 nm with respect to time. If $\log_{10}(E_t - E_f)$ is plotted against t (where E_t and E_f are the extinction at time t and the final extinction value respectively) a linear relationship is obtained. The gradient of the graph may be taken as an index of ribonuclease activity. The pH of each mixture was measured immediately after incubation. In these experiments the ionic strength was varied. In further experiments the ionic strength was maintained constant at the equivalent of 100 mM-NaCl and the relative proportions of NaCl, LiCl and $MgCl_2$ varied. Pancreatic ribonuclease activity was also assayed in the presence of sodium acetate buffer, pH 5·0 in a final concentration of 50 mM and the effects of the addition of various concentrations of NaCl, LiCl and $MgCl_2$ to a final ionic strength equivalent to that of 154 mM-NaCl studied.

Brain ribonuclease activity was assayed by a radioactive method which has been described in full elsewhere.[32] This involves preparing labelled rat brain RNA by injecting albino Wistar rats intraventricularly with 10 μCi [6-^{14}C]orotic acid, killing them 24 hr later and extracting the RNA by a modification (see below) of the phenolic

method of Scherrer and Darnell.[33] This labelled RNA, dissolved in 0·1 M-Tris–HCl buffer 8·1 served as substrate. Aliquots (0·25 ml) of the substrate solution (containing 140 μg RNA, specific activity 113 dis/min/μg) was added to 0·25 ml aliquots of rat brain homogenate of equal protein concentration (6 mg/ml) and incubated at 37° for periods of varying from 0 to 45 min. Control incubations were performed using 0·25 ml of Tris buffer instead of the tissue extract. The ionic composition of each series of incubations was varied in the same manner as described for the pancreatic ribonuclease assay. Each incubation was terminated by the addition of 50 μl 50% at 0°. Each tube was kept in ice for 5 min and then centrifuged. It was found that under these conditions there was a linear increase in the acid soluble dis/min and the rate of this increase expressed, per mg protein, gave a measure of the ribonuclease activity of the tissue extract. The protein in the acid insoluble precipitate was estimated by the Lowry method.[34]

Effect of chronic lithium administration on rat brain RNA metabolism in vivo. Clinically moderate amounts of lithium carbonate are given chronically to patients and the therapeutic effect does not become apparent until after a period of weeks. To stimulate the clinical situation male albino Wistar rats (weight 180–190 g) were injected intraperitoneally once a day for 13 days with a solution of lithium chloride (30 mg 3 ml in physiological saline) to give a daily dose of 0·15 mg/g. Control rats were given 1 ml physiological saline daily in a similar manner. Lithium chloride was used since the low solubility of lithium carbonate made it unsuitable for intraperitoneal injection. Intraperitoneal injections were necessary since rats failed to drink any water with lithium salts added. This dosage of lithium produced plasma levels equivalent to those obtained by the clinical dose given to humans. The plasma level of Li^+ in the treated rats estimated by flame photometry was 0·97 m-equiv./l. and the level in the cerebrospinal fluid was 0·31 m-equiv./l.

The rats had free access to water and rat pellets and their body weight and water intake was monitored over the 13 days. One hr after the intraperitoneal injection on the thirteenth day all the rats were injected intraventricularly (under light halothane anaesthesia) with 1 μCi [6-^{14}C]orotic acid and killed at intervals varying from 2 to 72 hr. The rats killed at 24, 48 and 72 hr are maintained on their daily injections of Li^+ or saline. The brains were removed and homogenized in 10 ml 6% trichloracetic acid (TCA) and 1 ml of the acid soluble fraction removed for determination of the acid soluble counts. The acid insoluble precipitate was washed twice with 5 ml 6% TCA and then defatted with one 5 ml aliquot of ethanol–diethyl ether (3 : 1 v/v) and three 5 ml aliquots of diethyl ether. All the above procedures were performed at 0–4°. The defatted acid insoluble precipitate was suspended in 5 ml of 0·3 N-NaOH and incubated at 37° for 18 hr. 0·5 ml of 50% TCA was then added at 0° and the mixture left for 30 min. The resulting precipitate was removed by centrifugation and 0·1 ml of the supernatant containing the product of alkaline hydrolysis of the brain RNA assayed by the orcinol method.[35] The radioactivity of the brain RNA was determined by counting 1 ml of the supernatant in a liquid scintillation spectrometer using a dioxane based scintillant. The acid soluble counts were determined in the same way. A DNA fraction was obtained by boiling the final precipitate with 5 ml 6% TCA for 15 min.

Effect of chronic lithium administration on the base composition of brain nuclear and cytoplasmic RNA. Experimental and control rats were subjected to the dose regimes

described above. On the thirteenth day the rats were killed and the brains removed and bissected. The total RNA of one hemisphere was extracted by the method of Scherrer and Darnell,[33] modified by deproteinizing with a mixture of phenol, *m*-cresol and 8-hydroxyquinoline in the following proportions: 500 g phenol, 70 ml *m*-cresol 55 ml H_2O and 0·5 g 8-hydroxquinoline. The second hemisphere was separated into the pure nuclear and cytoplasmic fractions the method described in the first paragraph of this section. The cytoplasmic fraction was composed of the first three 0·32 M-sucrose supernatants. The RNA was extracted from these fractions by the phenolic method outlined above.

The RNA was hydrolysed with 72% perchloric acid (PCA) at 100° for 1 hr (0·1 ml PCA/mg RNA). The resulting mixture of bases was separated by descending paper chromatography using Whatman No. 1 15 × 50 paper and a freshly prepared isopropanol/HCl solvent system. The chromatographic conditions and methods of calculating the number of μmoles of each base were identical to those described by Bendich.[36] The base composition was expressed in the form of the ratios: Adenine (A) + guanine (G)/cytosine (C) + uracil (U) (i.e. purine/pyrimidine) and A + U/G + C.

RESULTS

RNA polymerase activity in vitro. Li^+ could not replace Mg^{2+} as an essential co-factor for brain RNA polymerase. Figure 1 shows the result of incubating an "aggregate enzyme" preparation in media from which $MgCl_2$ was successively replaced by LiCl. No other monovalent cations were present. Significant polymerase activity was found only when 5 μmoles/ml $MgCl_2$ were present in the medium. Incubations from which Mg^{2+} was absent resulted in an incorporation of [^{14}C]GTP indistinguishable from that produced by the boiled enzyme control irrespective of the concentration

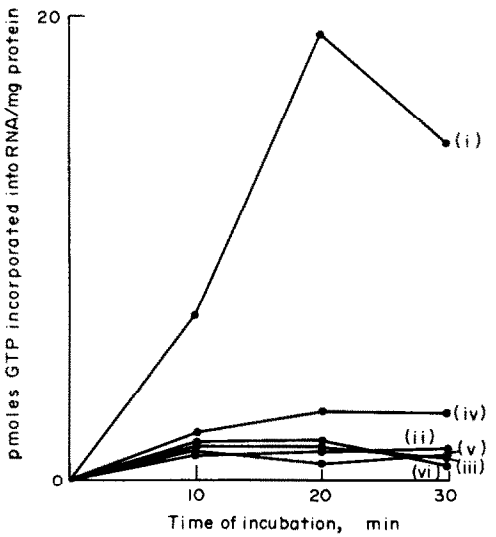

FIG. 1. The effect of lithium on the RNA polymerase 'aggregate enzyme preparation.' $MgCl_2$ omitted from medium unless stated otherwise. 30 min incubation, 37°. Experiment repeated three times. (i) Medium + 5 μmoles/ml $MgCl_2$; (ii) medium – no Li^+ nor Mg^{2+}; (iii) medium + 5 μmoles/ml LiCl; (iv) medium + 2·5 μmoles/ml $MgCl_2$ + 2·5 μmoles/ml LiCl; (v) boiled enzyme control; (vi) medium + 10 μmoles/ml LiCl.

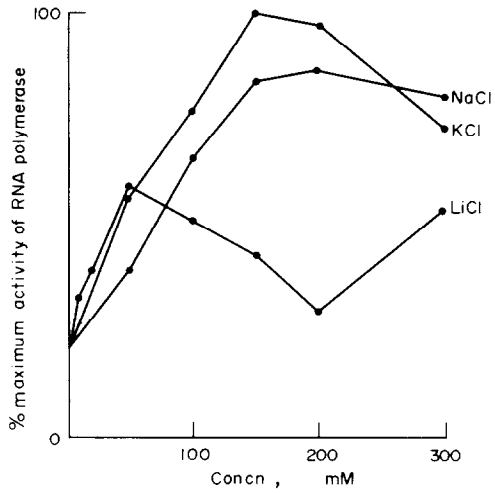

FIG. 2. The effect of Li^+, Na^+ and K^+ on the RNA polymerase activity of intact brain nuclei in the presence of 5 μmoles/ml $MgCl_2$. 15 min incubation 37° C. Number of experiments = 4.

of Li^+ present. Replacing half the $MgCl_2$ in the medium by an equimolar quantity of LiCl resulted in a very substantial reduction in polymerase activity.

Preliminary work with aggregate enzyme preparations indicated that in the presence of optimum concentrations of $MgCl_2$ (5 μmoles/ml) the addition of LiCl to the medium to a final concentration of up to 100 mM had a stimulatory effect and this was investigated more fully using intact nuclei. Figure 2 shows the result of incubating brain nuclei in media in which the $MgCl_2$ concentration was maintained at 5 μmoles/ml but the concentrations of LiCl, NaCl and KCl were varied from 0 to 300 mM. The results are expressed as a function of the maximum activity achieved with KCl in the medium. This was approx. 34 pmole ATP/mg DNA/15 min incubation. K^+ and Na^+ have basically the same effect, causing an enhancement of polymerase activity over a wide concentration range, the optimum being approx. 150 mM. The maximum activity achieved with Na^+ was 84 per cent of that achieved with K^+. Li^+ was less active and its effect was biphasic with respect to concentration. The maximum activity attained with Li^+ was approximately 60 per cent of that attained with K^+ but this maximum was reached at a lower concentration (approx. 50 mM). The addition of Li^+ to media containing both 5 μmoles/ml $MgCl_2$ and 150 mM KCl had no stimulatory effect.

The biphasic effect of Li^+ was not due to a difference in the response of neuronal and glial RNA polymerase. Both neuronal and glial nuclei showed a biphasic response to lithium and so did the RNA polymerase activity of liver nuclei (Fig. 3). Li^+ also had a biphasic effect on poly C synthetase activity (Fig. 4).

Ribonuclease activity. It was apparent from all experiments that Li^+ resembled Na^+ rather than Mg^{2+} in its effect on both pancreatic and brain ribonuclease. Figure 5(a) shows the activity of the former in the presence of single cations. Na^+ and Li^+ had a stimulatory effect at low concentrations (maximum activity at 40 mM) but this was not so apparent at higher concentrations. Mg^{2+} had a marked inhibitory effect at all concentrations studied. It is unlikely that these differences in activity could be accounted for by differences in pH. Na^+ and Li^+ had similar properties in buffered

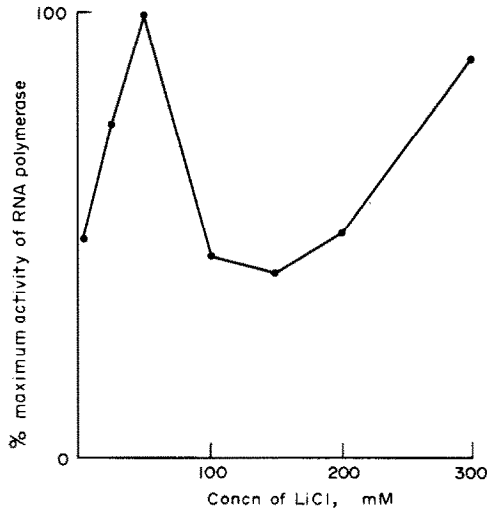

FIG. 3. The effect of Li^+ on the RNA polymerase activity of intact liver nuclei in the presence of 5 μmoles/ml $MgCl_2$. 15 min incubation, 37°. Number of experiments = 3.

assays and Mg^{2+} still showed a marked inhibitory effect on the enzyme activity. These results could not be explained by NaCl, LiCl and $MgCl_2$ having different effects on the u.v. spectrum of RNA. Comparison of the spectrum of RNA in distilled water and in 100 mM solutions of NaCl, LiCl and $MgCl_2$ revealed no shift in the spectrum that could result in a decrease in extinction at 300 nm.

Figure 6(a) shows the effect of Na^+, Li^+ and Mg^{2+} on brain ribonuclease activity determined by the ratioactive method at pH 8·1. Na^+ and Li^+ had a stimulatory effect at concentrations of up to 100 mM but thereafter had an inhibitory effect. Mg^{2+} had an inhibitory effect at all concentrations studied. A similar effect was observed at acid pHs but the inhibition by Na^+ and Li^+ at 200 and 300 mM was not so marked.

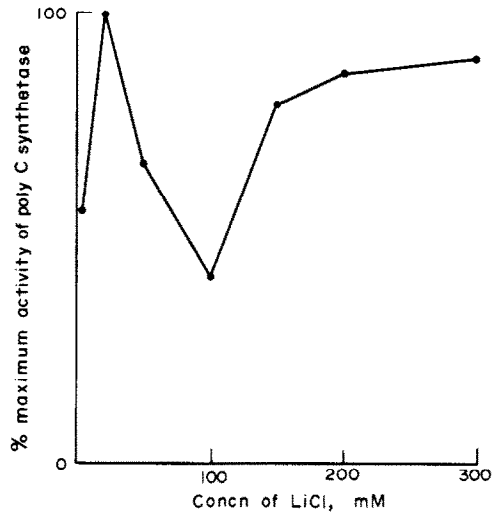

FIG. 4. The effect of Li^+ on the Poly C synthetase activity of intact brain nuclei in the presence of 5 μmoles/ml $MgCl_2$. 15 min incubation, 37°. Number of experiments = 3.

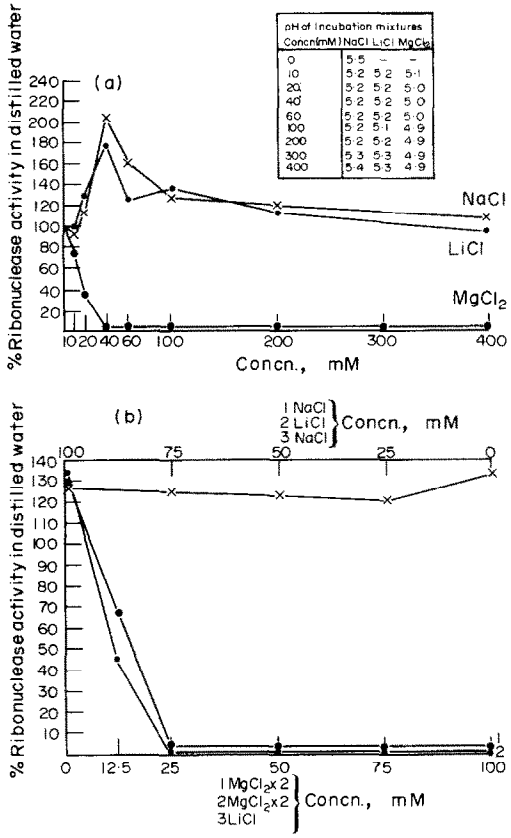

FIG. 5. The effect of NaCl, LiCl and $MgCl_2$ on pancreatic ribonuclease activity. (a) Varying the concentration of single cations; (b) varying the proportions of Na^+/Mg^{2+}, Li/Mg^{2+} and Na^+/Li^+; while maintaining constant ionic strength: no buffer added. number of experiments = 4.

The effects of altering the proportion of each ion while maintaining the ionic strength constant are shown in Fig. 5(b) (pancreatic ribonuclease) and Fig. 6(b) (brain ribonuclease). In both cases successive replacement of Na^+ or Li^+ by Mg^{2+} resulted in an inhibition of enzyme activity and it would appear that Li^+ does not reduce the inhibitory action of Mg^{2+}. Replacing Na^+ by Li^+ had little effect on pancreatic ribonuclease activity but rather curiously there was a slight reduction of brain ribonuclease activity when 40–60 per cent of the Na^+ had been replaced by Li^+. A similar picture was obtained with brain ribonuclease at acid pH. This type of experiment was repeated at an ionic strength equivalent to 154 mM-NaCl (physiological saline) but again replacing Na^+ by Li^+ had only a small effect on ribonuclease activity and replacing Na^+ or Li^+ by $Mg2^+$ resulted in a marked inhibition.

These results of a series of essentially "screening" experiments indicated that it is unlikely that lithium administration can cause an increased breakdown of RNA by a direct action on ribonuclease.

Studies in vivo. Rats subjected to lithium treatment invariably develop certain symptoms of lithium toxicity but the severity of these symptoms can vary considerably in different groups of rats. In the present study the rats exhibited polyuria in the

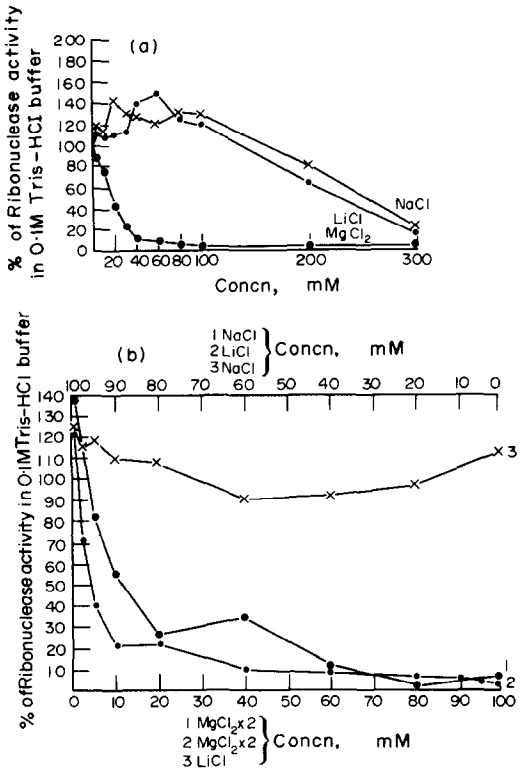

FIG. 6. The effect of NaCl, LiCl and $MgCl_2$ on brain ribonuclease activity at pH 8·1. Number of experiments = 2. (a) Varying the concentration of single cations; (b) varying the proportion of Na^+/Mg^{2+}, Li^+/Mg^{2+} and Na^+/Li^+ while maintaining constant ionic strength.

second week of treatment but did not suffer from any significant weight loss. At the commencement of the experiment the 18 rats in the control group weighed 183 ± 3 g and those in the lithium group 185 ± 3 g. After the 13 days of treatment the control group weighed 247 ± 5 g and the lithium group 243 ± 6 g. The water intake of the lithium-treated rats was approx. 65 ml/rat/day while that of the controls was 35 ml/rat/day. Although in this experiment lithium toxicity did not present any severe problems, in some preliminary investigations the lithium treated rats did suffer a marked weight loss and became emaciated in appearance. In a recent study on the effects of lithium administration on cerebral ATPase,* the symptoms of lithium toxicity have been largely eliminated by giving the animal access to a Minsal salt lick.

The content of nuclear and cytoplasmic RNA in the brains of lithium-treated rats did not differ significantly from controls, nor was there any indication from the base composition studies that there was any loss of purines or any other alteration in the composition of brain RNA (Table 1). The time course and extent of incorporation of [^{14}C]orotic acid into brain RNA did not differ in the lithium treated and control animals (Fig. 7). The labelling of DNA was almost negligible and did not differ in the two groups of animals. Only a small fraction (approx. 8 per cent) of the intraventricularly administered radioactivity was recovered in the brain. However this

* A. J. Dewar, N. Kinloch and H. W. Reading, manuscript in preparation.

TABLE 1. THE RNA CONTENT AND BASE COMPOSITION IN THE BRAINS OF RATS TREATED CHRONICALLY WITH 0·15 mg/g LiCl DAILY

RNA-type	Animals	RNA content (mg/g wet wt brain)	$\frac{A + G}{C + U}$	$\frac{A + U}{G + C}$
Total RNA	Control	2·61 ± 0·05 (24)	1·145 ± 0·026 (6)	0·733 ± 0·032 (6)
	Lithium-treated	2·58 ± 0·04 (24)	1·153 ± 0·030 (6)	0·721 ± 0·019 (6)
Nuclear RNA	Control	0·24 ± 0·008 (6)	1·017 ± 0·053 (6)	0·941 ± 0·042 (6)
	Lithium-treated	0·23 ± 0·007 (6)	1·032 ± 0·051 (6)	0·962 ± 0·050 (6)
Cytoplasmic RNA	Control	2·39 ± 0·06 (6)	1·149 ± 0·046 (6)	0·729 ± 0·042 (6)
	Lithium-treated	2·31 ± 0·07 (6)	1·142 ± 0·042 (6)	0·741 ± 0·039 (6)

Values represent mean ± S.E.M. *n* appears in parentheses.

value compared favourably with recoveries obtained using other methods of intracerebral injection.[22,37]

DISCUSSION

The results of the *in vitro* study of the effects of lithium on RNA polymerase and ribonuclease do not suggest that lithium, at the concentrations encountered therapeutically, could affect cerebral RNA turnover by a direct action on these enzymes. The only interesting feature of the action of lithium was its biphasic

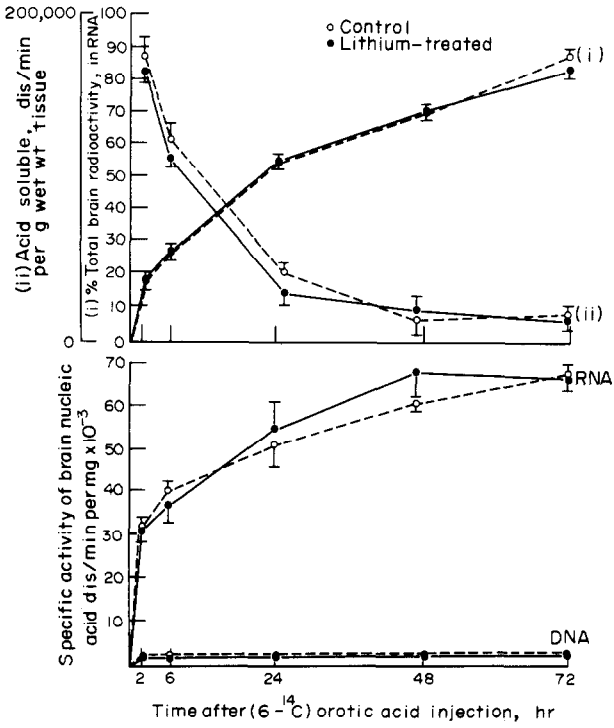

FIG. 7. The effect of prolonged administration of 0·15 mg LiCl/g/day on the incorporation of [6-^{14}C]-orotic acid into the brain RNA and DNA of albino Wistar rats. Each value represents the mean of four rats. Bar lines represent S.E.M.

stimulatory effect on RNA polymerase and Poly C synthetase. The monophasic effect found with sodium and potassium resembled that found by previous workers.[19] A biphasic stimulatory effect on RNA polymerase has also been demonstrated with ammonium ions[38] but this was at a slightly higher ionic strength. It is unlikely that the biphasic effect of lithium has any clinical significance since the concentrations required are above those encountered therapeutically.

It is known that there are two main types of RNA polymerase, one active at low ionic strength, activated by Mg^{2+} and believed to synthesize r. RNA, and the other active at high ionic strength activated by Mn^{2+} and NH_4^+ and believed to synthesize a more DNA, like RNA.[39] The present study has been concerned with the former type for two reasons: any changes in RNA metabolism that account for the change in uric acid levels would have to involve ribosomal RNA and Li^+ resemble Mg^{2+} in some of its properties (see Introduction).

Although the studies *in vitro* proved negative, the number of effects claimed for lithium by other workers suggested the possibility that an indirect effect on RNA metabolism could result from chronic *in vivo* administration of lithium. In particular its reported effects on energy metabolism[40,41] and on magnesium and calcium distribution[42,43] might be expected to have repercussions on cerebral RNA metabolism especially since it has been suggested that calcium ions play a crucial part in linking cerebral activity with intracellular RNA and protein metabolism.[43]

The present *in vivo* results, however, do not bear out this supposition and lithium did not appear to significantly alter cerebral RNA metabolism even by indirect means. This disagrees with our previous finding[45] that there was a transitory increase in the labelling of RNA in the brain but not in the liver. However, in the previous study the weights of the rats on lithium treatment were not monitored nor was there any determinations of the acid soluble precursor pool. It is probable that the earlier finding was a precursor effect. The problems involved in assessing brain RNA synthesis have been discussed elsewhere.[32,37] The present results provide no evidence for any breakdown of brain RNA resulting from lithium administration nor is there any evidence for an alteration in its composition.

On the basis of these results it is unlikely that the phasic changes in uric acid excretion observed in manic-depressive patients[11] reflect changes in cerebral RNA metabolism. It is also unlikely that they reflect changes in the RNA metabolism of any other tissue. Chronic lithium administration has no effect on the RNA metabolism in the liver[45] and in a study of RNA metabolism in the leucocytes of manic depressive patients no phasic changes associated with the phase of the illness were observed.[46]

The changes in uric acid excretion could be due to a uricosuric effect or may reflect changes in the metabolism of purines. Recently marked changes in blood ATP have been observed in manic depressive patients[47] and in preliminary experiments[46] we have shown that chronic lithium administration in rats resulted in a reduction in the total brain adenine nucleotides from 152 μmoles/100 g wet wt to 136 μmoles/100 g wet wt. However, further studies on rats are of limited usefulness due to the fundamental difference in the purine catabolic pathways of rats and primates, the former excreting negligible uric acid.

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