Biochemical Studies on the Effects of Lithium Salts: Excretion of Tricarboxylic Acid-Cycle Intermediary Metabolites, Inhibition of Vasopressin and Distribution of other Ions in the Body

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We have demonstrated that, at least in one well-controlled patient, with a manic-depressive illness with an unusual but precise 48h rhythm, the process could be stopped and re-started by altering the Li+/Na⁺ ratio of the diet (Hanna et al., 1972). Treatment was effective after 18 years of severe illness, and for 4 years the patient remained well; nevertheless the 48h rhythm of illness returned when lithium salt was replaced by a placebo, as was shown in his mood, his urine volume, salivation rate etc. Following this, the work of the Medical Research Council Unit for Metabolic Studies in Psychiatry included studies of the physical effects of lithium salts. The clinical results, though striking, are simply a confirmation of other studies (e.g. Coppen et al., 1971; Baasstrup et al., 1970).

Since these studies our work on the effects of treatment with lithium salts has been directed in several directions, including studies of the effects of the treatment on: (i) the excretion of organic acids and particularly tricarboxylic acid-cycle intermediates; (ii) the action of vasopressin; (iii) the redistribution of other ions in the body; (iv) the resting and the sleeping electroencephalogram; (v) urine amines and steroids. Some findings on (i)-(iv) are presented here.

Excretion of organic acids

Bond et al. (1972) demonstrated that lithium salts in therapeutic doses increase the renal excretion of 2-oxoglutarate and glutarate, but not of citrate, pyruvate or lactate, in both normal and manic-depressive men. The fact that increased 2-oxoglutarate excretion is not accompanied by increased citrate excretion (as can be produced by NaHCO₃) shows that the phenomenon is not a simple response to the alkaline urine produced by the administration of lithium salts. The titratable acidity of urine, however, does fall on the first day of therapy with lithium salts. This is largely due to an increased excretion of HCO₃⁻ ions. Almost identical responses are given by LiCl and by Li₂CO₃, but it is necessary to give more than 6mequiv. of Li⁺ twice a day to detect a clear effect in 24h urine samples. All persons studied with the usual clinical doses of 18–36mequiv. of Li⁺/day for the carbonate, giving plasma concentrations of approx. 0.5–1.5 mequiv./l, showed quite clear responses. There was, however, much variation in the timing of the response and of the amplitudes achieved. The amount of 2-oxoglutarate lost increases slowly over several months of treatment, but nevertheless bears a close relationship during the day to the amount of Li⁺ also excreted, and on cessation of treatment both fall together with a somewhat similar timing.

In contrast, excretion of HCO₃⁻ is raised only on the first day of treatment; on a controlled diet it subsequently returns to control values, but on the day after cessation of the treatment with lithium salt a compensatory fall in HCO₃⁻ excretion occurs for one further day. Hence in some sort of way an equilibrium state is produced and maintained as long as lithium salt is given at the same dose. What this compensatory state is important in affecting is still a matter for speculation, as is its nature. Perhaps mitochondrial binding is involved (see Gear & Lehninger, 1968).

Within the limits of the method used (Bergmeyer & Bernt, 1963) there is no discernible change of plasma 2-oxoglutarate concentration, nor of plasma alkali reserve (after the administration of lithium salts). The considerably diminished renal clearance of 2-oxoglutarate therefore seems consistent with an inhibition of net tubular reabsorption. The increased excretion of glutarate and of succinate, adipate, fumarate, α-hydroxyglutarate and 2-hydroxy-4-oxoglutarate more recently demonstrated in this Unit (Lee & Pollitt, 1973) after the administration of lithium salts may be seen as consistent with this view. They suggest that structural relationship is more important than
metabolic relationship. On the other hand, as fumarate, succinate and 2-oxoglutarate are all excreted in increased amounts, but not citrate, pyruvate or lactate, a direct effect of the ion on enzymic processes in the tricarboxylic acid cycle cannot be excluded.

Concomitantly, and in order to explore the effect of treatment with lithium salts on the excretion of 2-oxoglutarate further, P. A. Bond, L. Grant & F. A. Jenner (unpublished work) have studied rats injected with 0.2mmol of the chloride or citrate of Li⁺ or other ions intraperitoneally. The urine 2-oxoglutarate and citrate were measured in the 3h before the injection and compared with those in the 3h after the injection. Rats, like men, show a clear increase of urine 2-oxoglutarate both in absolute amounts and measured as concentrations. Similar injections of the chlorides NaCl, RbCl, CsCl, MnCl₂, MgCl₂, KCl and SrCl₂ do not produce such an increase. There is a small circadian increase, and also sodium citrate causes an increase, possibly due to the alkalotic effect. Mn²⁺ was too generally toxic to the animals, but the animals' behaviour and general condition were not obviously affected by the other ions. Cs⁺ and Ca²⁺, and to a smaller extent Rb⁺, have the opposite effect to Li⁺, and decrease 2-oxoglutarate excretion after acute administration. Fig. 1 summarizes the results obtained. Under the conditions of the study, only Li⁺ and Mg²⁺ appear in the 3h urine as a significant proportion of the injected material. However, though this might suggest that the effect is bound to the presence of the foreign cation in the urine or kidney, studies involving chronic treatment with Rb⁺, Cs⁺ or Li⁺ in the food until similar urinary excretions are produced does not

Fig. 1. Acute effects of intraperitoneal injections on 2-oxoglutarate excretion
2-Oxoglutarate was measured in rat urine during the 3h preceding intraperitoneal injection of the salts and for 3h after the injection. Animals received 0.2 mequiv. of the cation unless another value is quoted. The lines represent the log of the mean ratio ± S.E.M. of the 2-oxoglutarate excretions before and after the injection, presented in terms of grams excreted (●) and of concentration (▲).
substantiate that suggestion. On the contrary, it reveals that Rb⁺ and Cs⁺ continue to suppress 2-oxoglutarate excretion. Considerable further effort is being devoted to the effects of Li⁺ on the tricarboxylic acid-cycle intermediates and related compounds in other tissues, as well as in the kidney and urine, but few results are currently available. However, it has not proved possible in the rat brain to confirm the finding that Li⁺ lowers the activity of succinate dehydrogenase in the mouse brain, reported by Abreu & Abreu (1972) (P. A. Bond, L. Grant & F. A. Jenner, unpublished work).

The possibility that our essentially renal studies might be analogues of more important studies in the nervous system seems to be increased by the earlier results obtained by Berl & Clarke (1971) and DeFeudis & Delgado (1970), who both in effect show that Li⁺ tends to lead to a decrease in brain glutamate. This also highlights the possible relevance of the work of, for example, Wiechert & Knaape (1972) on the cerebral activity of the derivatives of glutamate.

Inhibition of vasopressin

By using the preparation described by Bisset (1962), the ethanol-anaesthetized and water-loaded rat for the assay of vasopressin, it was found that Li⁺ is a specific and reversible inhibitor of vasopressin (Harris & Jenner, 1969a,b). Li⁺ (13 mequiv./l) was given intravenously in an infusion in which Na⁺ (13 mequiv./l) was replaced in a modified Czaczkes et al. (1964) solution. Na⁺, K⁺, Ca²⁺, Mg²⁺, Sr⁺ and Rb⁺ show similar inhibition when given in comparable amounts. It was, however, also noted that it was necessary to administer continuously the Li⁺ to sustain the inhibition. The inhibition was quickly reversed, despite high plasma concentrations of Li⁺, 30 min after the administration of Li⁺ had been stopped. This shows clearly that neither the absolute concentrations of whole-body Li⁺ nor those of plasma Li⁺ are critical for the inhibition, and suggests that the effect depends on the intracellular/extracellular Li⁺ concentration ratio (Harris & Jenner, 1971a). It is equally noteworthy that, though Rb⁺ and Sr⁺ do not affect the action of vasopressin when being administered, they do seem to increase the sensitivity of the preparation when one stops giving them (Harris & Jenner, 1972), perhaps when they are leaving the 'cells'.

The production of a vasopressin-resistant diabetes-insipidus-like state by treatment with lithium salts is well recognized both clinically (Angrist et al., 1970) and in rats (Schou, 1958; Thomsen, 1970). Whether our results are relevant to this is at least open to question, as clinically it follows chronic administration, but possibly occurs when the hypothalamus can no longer compensate for the renal inhibition. However, our own preliminary results in man do not show a rise in vasopressin excretion after treatment with lithium salts.

Inhibition of adenylate cyclase has been suggested as a possible explanation of the inhibition of vasopressin produced by treatment with lithium salts (Dousa & Hechter, 1970; Forn & Valdecasas, 1971). Harris & Jenner (1971b) have, however, shown that Li⁺ inhibits the water transport facilitated by cyclic AMP as well as that facilitated by vasopressin. Both in the rat kidney and in the toad bladder Li⁺ also inhibits the vasopressin-like effects on water of oxytocin (Harris & Jenner, 1972).

In further work in this Unit with toad bladders, Li⁺ at 2 mequiv./l (E. A. Thompson, unpublished work) did not inhibit Na⁺ transport facilitated by vasopressin measured as the short-circuit current, nor did it affect the resting potential difference. At higher concentrations (10 mequiv./l) the short-circuit current is profoundly lowered. This might suggest that the important therapeutic action could be unrelated to effects on Na⁺.

The study of the inhibition of vasopressin by treatment with lithium salts requires further study. Goodwin & Jenner (1967) have shown that the antidiuretic activity of urine can change with changing mood in periodic psychosis. Vasopressin also has a behavioural effect, at least in rats, as it decreases the rate of extinction of conditioned avoidance learning (De Wied, 1969). Preliminary studies show that lithium salts, far from inhibiting this effect, act like vasopressin in the rat, but also cause an increase in serum vasopressin concentration (E. A. Thompson & D. De Wied, unpublished work).
work). In the rat this could be due to the hypothalamic compensation for the renal inhibition, but a direct effect of Li\(^+\) on the hypothalamus also seems likely, and is supported for the drinking centres of the lateral hypothalamus by the result obtained by Smith \textit{et al.} (1971).

\textit{Distribution of cations in the body}

The administration of lithium salts is clearly the cause of multiple disturbances and interactions with the distribution of ions other than Li\(^+\). Birch (1971) has shown that in rats given lithium salts in their drinking fluid (8 mequiv. of Li\(^+/l\)) or injected intraperitoneally daily with 0.2 mequiv. of Li\(^+\) as the citrate or chloride for 28 days there is a decrease in brain Na\(^+\) and Mg\(^{2+}\), bone Na\(^+\) and Ca\(^{2+}\) and urine K\(^+\) and Na\(^+\); at the same time there is an increase in muscle Ca\(^{2+}\), plasma Mg\(^{2+}\), urine volume and renal Ca\(^{2+}\) excretion. Li\(^+\) itself is specially concentrated in bone.

Hullin \textit{et al.} (1968) had already shown that there is a positive Li\(^+\) balance for some time in patients treated with Li\(_2\)CO\(_3\). After the treatment had ceased, they showed episodes of Li\(^+\) excretion still occurring. This might be explained, at least in part, by some laying down of Li\(^+\) in bone.

\textit{Electroencephalographic studies}

In studies on the electroencephalograms of human subjects, we have found amplitude increases and slowing of the frequencies seen in some persons as a consequence of treatment with lithium salts (see also Helmchen \& Kanowski, 1971) that can persist for up to 5 weeks after its administration has been discontinued (see Fig. 2). On simply decreasing the dose the electroencephalographic changes have occasionally been found to be a good early indication of impending relapse (see Hanna \textit{et al.}, 1972). If these electroencephalographic changes are very directly related to the therapeutic effect of treatment with lithium salts, their timing would suggest that many of the other factors studied in our work may be fairly irrelevant to therapy. However, much more work is required to explore the implications of these and other people’s studies.

\textit{Discussion}

The above alone might warn us against trying to fit all the effects of treatment with lithium salts into one hierarchy of events. However, the clear interactions between carbohydrate metabolism, Mg\(^{2+}\) and Ca\(^{2+}\), and the importance of the latter for the action of vasopressin, give some grounds for speculation. Li\(^+\) has a valency and charge like that of Na\(^+\) and K\(^+\), but an ionic potential like that of Ca\(^{2+}\) and an ionic radius like that of Mg\(^{2+}\) (see Birch, 1970). Carbohydrate metabolism is affected, and clinically this can be shown by the increased mean blood glucose concentrations after treatment with lithium salts and increased concentrations in glucose tolerance tests (Shopsin \textit{et al.}, 1972). Li\(^+\) might do this by stimulating the effect of hexokinase (Balan \textit{et al.}, 1970), and this may be related to the increased glucose uptake of tissues after treatment with lithium salts (Bhattacharya, 1959).

Li\(^+\) also inhibits pyruvate kinase (Balan \textit{et al.}, 1970), but our failure to detect any change in the urinary excretion of pyruvate, lactate and citrate in man, despite clear changes in that of other tricarboxylic acid-cycle metabolites, suggests this might be only causing a comparatively minor disturbance. The inhibition by Li\(^+\) of protein kinase (Walaas \textit{et al.}, 1970) also seems remote from our findings. The field is well reviewed by Mellerup \textit{et al.} (1973), which group has also shown that LiCl injections in rats cause an increase of a radioimmunoassayable glucagon-like substance in plasma (Mellerup \textit{et al.}, 1970). Although these authors say this is a peripheral effect, it could be of central importance. They have subsequently also shown that there is an increase in serum insulin concentration after treatment with lithium, and this is possibly secondary to the glucagon rise (E. T. Mellerup, H. Gronland Thomsen, N. Bjørn & O. J. Rafaelsen, personal communication), which they suggest may be related to the increases in body weight described by Kerry \textit{et al.} (1970) in patients treated with lithium salts. This increase in body weight due to the accumulation of fat may have to be distinguished from changes in the volume of body water also occurring in these situations.
On \( \text{Li}_2\text{CO}_3 \) (500 mg twice daily),

\[ \text{Fig. 2. Typical electroencephalographic traces from a patient before receiving } \text{Li}_2\text{CO}_3, \text{ during } \text{Li}_2\text{CO}_3 \text{ therapy and 5 weeks after receiving } \text{Li}_2\text{CO}_3. \]

The persistence of an increased amplitude, slowing and other abnormalities can be seen.


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Animal Models for Behavioural and Biochemical Studies on the Effects of Lithium Salts

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The aim of our work has been to induce in laboratory animals forms of behaviour that resemble aspects of human manic-depressive illnesses and to determine ways in which such forms of behaviour can be modified by lithium salts and by other drugs. Eventually we hope to relate these behavioural changes to changes in concentrations of brain amines.

We have found that the behaviour of animals whose activity is artificially increased by the administration of drug mixtures and by other means is dramatically altered by pretreatment with relatively small doses of lithium salts. Lithium salts abolish the hyperactivity, and the behaviour of the rats and mice appears to be 'normalized'. Lithium salts by themselves had no effect on the activity of saline-treated controls, and did not diminish the activity of animals treated with the separate drugs and which were not hyperactive. It thus appears that treatment with lithium salts can specifically prevent animals from becoming hyperactive, without affecting their normal behaviour.

Lithium salts have been reported to be effective in the treatment of mania (Cade, 1949; Schou, 1959, 1968; Maggs, 1963; Pearson & Jenner, 1971) and in the prophylaxis of recurrent manic-depressive episodes and mood swings (Schou, 1968; Angst et al., 1970; Coppen et al., 1971). However, there have been few pertinent studies on the effect of lithium salts on the behaviour of laboratory animals. Cade (1949) noted lethargy in guinea pigs. Matussek & Linsmayer (1968) found that the administration of lithium salts diminished a form of motor hyperactivity induced by a combination of demethyl-imipramine and compound Ro-1284 (a reserpine-like drug), but had no effect on hyperactivity induced by amphetamine alone. The administration of lithium salts has