Br. J. Pharmac. (1974), **50**, 283-289

THE EFFECT OF LITHIUM AND RELATED METAL IONS ON THE URINARY EXCRETION OF 2-OXOGLUTARATE AND CITRATE IN THE RAT

P.A. BOND & F.A. JENNER

Medical Research Council Unit for Metabolic Studies in Psychiatry,
University Department of Psychiatry, Middlewood Hospital, P.O. Box 134, Sheffield, S6 1TP

1 Administration of lithium ions to rats, either acutely by intraperitoneal injection or chronically in food, causes increased excretion of 2-oxoglutarate and citrate.

2 Chronic administration in food of rubidium and caesium causes decreased excretion of 2-oxoglutarate and citrate.

3 The effects described are not due to changes in urine volume, nor pH, nor are they simply related to the excretion of the injected ion.

4 Acute administration of lithium caused an increased level of 2-oxoglutarate in kidney and reduced the ratio of glutamate to 2-oxoglutarate.

5 Renal gluconeogenesis in slices was only slightly affected by either acute administration of lithium to the animals or by its presence in the incubation medium of renal slices.

Introduction

We have previously shown that in therapeutic doses, used in manic depressive psychoses, lithium causes in man an increased renal excretion of 2-oxoglutarate and glutarate (Bond, Jenner, Lee, Lenton, Pollitt & Sampson, 1973). The possibility that this is relevant to the therapeutic and behavioural effects (see e.g. Platman, 1971, Schou, 1968) of lithium has led us to explore the phenomenon in more detail. In this study, the particular questions posed are: (a) is the rat a suitable model; (b) to what extent is the effect specific to lithium; (c) is there a change in tissue concentrations; (d) is there an effect on gluconeogenesis?

Methods

In studies of salt administration male rats were used (CFY strain, Carworth Europe, Alconbury, Huntingdon), aged 42 days on arrival and weighing 0.21 kg. Each batch was randomly split into two groups of three animals, placed in ordinary cages in the experimental environment and subjected to a reversed light cycle of 12/12 L.D. for four days. Each animal was then put into an all-glass Jencon type metabolic cage (Howells, Wright & Harrison, 1964) and given food and water *ad libitum*. Those to receive intraperitoneal injections did so at 13 h 00 min each day for five consecutive experimental days. Urine was collected from 10 h 00 min to 13 h 00 min and then from 13 h 00 min to 16 h 00 min. Creatinine content was measured in each urine and the results were considered in terms of creatinine excretion; but as this produced results similar to those expressed per unit time, these measurements are not included in the results section. Chronically dosed animals were given various salts added to the food (Carworth Dixon Diet) which was then made into a slurry by adding an equal weight of water to ground diet. Unless otherwise stated, batches of animals were six in number.

Chemical estimations in urine were made as follows: metal cations by atomic absorption spectroscopy, except for sodium and potassium which were assayed by flame photometry; urine titratable acidity with an automatic titrator (Radiometer, Copenhagen); creatinine and bicarbonate by methods recommended for a Technicon Autoanalyser; citrate by the method of Beutler & Yeh (1959); 2-oxoglutarate by the method of Bergmeyer & Bernt (1963).

Measurement of gluconeogenesis was carried out with slices of rat kidney. Male rats (0.15-0.2 kg) were killed by cervical dislocation and both kidneys rapidly removed. Slabs of cortex (200-400 mg) were cut at 0.2 mm intervals with a McIlwain tissue chopper (Mickle Laboratory Engineering Company, Gomshall, Surrey) in two directions at 45°. The resulting prism shaped slices

(0.2 x 0.2 x approx. 2 mm) were suspended in 5 vols of ice-cold incubation medium of Robinson (as described in Umbreit, Burris & Stauffer, 1957) without glucose. Portions of suspension (0.25 ml containing 50 mg of tissue) were pipetted into 25 ml conical flasks containing 3.70 ml of incubation medium (previously oxygenated). The flasks were gassed with oxygen, sealed with Parafilm (Gallenkamp Ltd) and incubated in a shaking water bath at 37° C for 15 minutes. The substrate (in 50 μl) was then added to the flask and the incubation was continued for 60 minutes. When oxygen uptake was to be measured simultaneously, incubations were carried out at 37° C in a Warburg respirometer (Umbreit *et al.*, 1957). The tissue slices were then separated by filtration under vacuum by means of a small Buchner funnel fitted with a Whatman No. 1 filter disc (22 mm diameter). Glucose was estimated in the filtrate essentially by the hexokinase method described for use with the Biochemica test combination for blood glucose (Boehringer Mannheim GMBH). The enzymes used were hexokinase, type F-300, 350 units/mg protein, and glucose-6-phosphate dehydrogenase type XII from Torula yeast, 400 units/mg protein (both from Sigma Ltd).

Levels of 2-oxoglutarate and glutamate in kidney were estimated by a freeze clamp technique (Hess, 1963). The two metabolites were measured spectrophotometrically in the neutralized perchloric acid extracts using glutamate dehydrogenase. In the 2-oxoglutarate assay the following were put into a 2 cm semi-microcell: 0.5 ml of neutralized perchloric acid extract (or 0.02 ml of urine), 0.025 ml of NADH (2.0 mg/ml), 0.1 M phosphate buffer (pH 7.6) to 1.975 ml; the optical density at 340 mμ was read and 0.025 ml of glutamate dehydrogenase (Type I-ammonium sulphate suspension diluted to 2 mg protein/ml with 2.8 M ammonium sulphate, Sigma Ltd) was added. The reaction was allowed to reach completion (usually 3-4 min) when the optical density was again read. A standard solution of 2-oxoglutarate was added to the cells and after 3-4 min a further reading was taken. The optical density change due to this addition of 2-oxoglutarate was the same as that obtained with aqueous standards run through the assay procedure. For the glutamate assay the following were put into a 2 mm semi-microcell: 0.1 ml of neutralized perchloric acid extract, 0.1 ml NAD (70 mM) and 0.5 M glycine buffer (pH 9.0) containing 0.4 M hydrazine sulphate to 1.98 ml (Sigma Ltd). The optical density at 340 mμ was read and 0.02 ml of glutamate dehydrogenase (type II, from bovine liver in sodium phosphate and 50% glycerol, Sigma Ltd) was added. The optical density was measured again after 45 minutes. The change in optical density was used to calculate the glutamate concentration by means of a standard curve. The standard curve allows for the increase in optical density due to the interaction of NAD with the hydrazine in the buffer.

The statistical method used was Student's *t* test (Fisher, 1954). Values are given with standard errors where appropriate. The following abbreviations are used: N = number of animals; *n* = number of estimations; s.e. mean = standard error of the mean, or s.d. = standard deviation.

Results

Table 1 shows the effects of various salts (injected intraperitoneally) on the urinary excretion of 2-oxoglutarate and citrate. The results are presented as ratios of concentrations and of absolute amounts excreted in the 3 h following the injection, to those excreted in the 3 h preceding the injection. The use of ratios shows the consistency of rises or falls and allows Student's *t* test to be employed appropriately. Studies of mean values of groups of animals before and after injection precludes the appropriate use of such a parametric test, as the variances are unsatisfactory, due to the variability of 2-oxoglutarate and citrate excretion. The *P* values obtained might therefore be misleading. The mean pretreatment values for 2-oxoglutarate were, nevertheless: 0.90 ± 0.55 s.d. mg/3 h and 0.257 ± 0.158 s.d. mg/ml. After treatment with 0.2 mM lithium chloride they were: 4.22 ± 1.40 s.d. mg/3 h and 0.60 ± 0.28 s.d. mg/ml. For citrate, initial means were: 3.76 ± 3.5 s.d. mg/3 h and 0.96 ± 0.43 s.d. mg/ml; and after treatment with 0.2 mM lithium chloride: 8.35 ± 3.07 s.d. mg/3 h and 1.17 ± 0.50 s.d. mg/ml.

For 2-oxoglutarate, 29 out of 30 animals which received 0.2 mM lithium chloride showed an increase in concentration and amount; and for the odd one, the concentration alone rose. For citrate, 23 out of 30 animals showed an increased concentration of lithium chloride while 28 out of 30 animals excreted increased amounts.

Lithium citrate injection led to the highest increases, though lithium chloride, and to a lesser extent sodium citrate, increased 2-oxoglutarate and citrate excretion. Injection of the chlorides of sodium, potassium, rubidium, caesium, strontium and magnesium tended to decrease 2-oxoglutarate and citrate concentration in urine, hence ratios below one were obtained. If sodium chloride (0.2 mM) is treated as the control, which takes into account effects of time of day and of injection, then calcium and caesium cause highly significant decreases in 2-oxoglutarate and citrate

Table 1 The effect of acute injections of lithium and other metal salts on the urinary excretion of 2-oxoglutarate and citrate.

Salt	No. of injections	2-oxoglutarate		Citrate	
		Mean of the ratios (totals mg/3 h)	Mean of the ratios (conc. mg/ml)	Mean of the ratios (totals mg/3 h)	Mean of the ratios (conc. mg/ml)
Lithium citrate 0.2 mEq	20	9.01 ± 1.545	5.82 ± 0.924	4.56 ± 0.409	3.11 ± 0.566
Sodium citrate 0.2 mEq	20	2.76 ± 1.100	1.45 ± 0.233	2.08 ± 0.321	1.37 ± 0.097
Sodium chloride 0.1 mM	15	1.16 ± 0.159	1.05 ± 0.148	1.27 ± 0.264	1.06 ± 0.210
Sodium chloride 0.2 mM	36	1.08 ± 0.064	0.88 ± 0.039	1.11 ± 0.080	0.95 ± 0.082
Lithium chloride 0.05 mM	6	2.45 ± 0.265	3.31 ± 0.840	0.98 ± 0.097	1.23 ± 0.171
Lithium chloride 0.1 mM	9	5.76 ± 2.515	2.44 ± 0.368	1.75 ± 0.285	1.15 ± 0.134
Lithium chloride 0.2 mM	30	6.01 ± 0.619	2.87 ± 0.308	2.86 ± 0.262	1.44 ± 0.11
Magnesium chloride 0.2 mM	6	0.90 ± 0.135	0.91 ± 0.092	0.91 ± 0.092	0.76 ± 0.068
Calcium chloride 0.2 mM	12	0.37 ± 0.058	0.43 ± 0.057	0.38 ± 0.039	0.54 ± 0.043
Rubidium chloride 0.2 mM	12	0.85 ± 0.081	0.91 ± 0.113	0.70 ± 0.052	0.72 ± 0.034
Strontium chloride 0.2 mM	6	1.04 ± 0.176	0.69 ± 0.090	0.86 ± 0.211	0.57 ± 0.131
Caesium chloride 0.2 mM	6	0.53 ± 0.074	0.38 ± 0.051	0.77 ± 0.080	0.55 ± 0.067
Potassium chloride 0.2 mM	6	1.24 ± 0.232	0.78 ± 0.086	1.27 ± 0.236	0.78 ± 0.076
Manganese chloride 0.1 mM	3	0.34 ± 0.189	0.53 ± 0.063	0.53 ± 0.205	0.66 ± 0.059
Manganese chloride 0.2 mM	6	0.57 ± 0.300	1.16 ± 0.090	0.30 ± 0.087	0.79 ± 0.164

Values are expressed as a ratio of the levels in the 3 h following and preceding an injection (i.p.) of the appropriate salt.

both in urine concentration as well as in absolute amounts excreted. Rubidium produces a less convincing decrease, which in acute studies is statistically significant ($P < 0.125$) for the absolute amounts of 2-oxoglutarate excreted but not for the urine concentrations. For citrate obvious decreases occurred following rubidium.

The citrates of lithium and sodium cause more profound increases than their chlorides in part because they lead to the production of alkalotic urine, a factor well known to increase organic aciduria (see Crawford, Milne & Scribner, 1959).

The situation is however somewhat more complicated as lithium chloride injections cause a rise in urine pH due to increased bicarbonate excretion (Jenner, 1973).

Further, from Table 2, which shows the amounts of injected ion recovered in urine passed during the 3 h following the injection in the above experiments, lithium is treated very differently from many other foreign ions in being excreted more rapidly. The time course of the increased 2-oxoglutarate excretion in the studies was also similar to that of the lithium excretion. It could therefore be that the organic acids are excreted to neutralize the charge of the foreign ion in the urine. In Table 3, however, results are presented under conditions in which the urine content of the lithium, caesium and rubidium, are made more comparable. This was achieved in chronic experiments by feeding the ions in the food until the quoted levels (Table 3) were produced in the urine. Lithium, N = 6, $n = 23$, still caused an increase in urine 2-oxoglutarate ($P < 0.001$) compared to animals receiving food with the equimolar addition of sodium chloride to the diet. For citrate excretion, however, a significant increase is only achieved for the absolute amount excreted and not for the urine concentration. This could therefore in this situation be partly dependent on urine volume changes, though these are very small. Caesium (N = 5; $n = 20$) and rubidium (N = 5; $n = 19$) cause very much more pronounced

Table 2 Urinary excretion of some metal ions in the 3 h preceding and following a single injection (i.p.) of 0.2 mM of the chloride.

	Output (μM ± s.e. mean) per 3 h	
	Before injection	Following injection
Lithium	0	64 ± 4.5
Rubidium	0	7.7 ± 0.9
Caesium	0	11.8 ± 0.9
Magnesium	33 ± 4.4	89 ± 20
Calcium	2.5 ± 0.73	9.3 ± 1.9

Table 3 Urinary excretion of 2-oxoglutarate and citrate and titratable acidity in rats given lithium sodium, rubidium or caesium in the diet.

Ion	Urine levels of administered ion (mEq/24 h)	Mean pH	Mean titratable acidity (mEq)	2-oxoglutarate Conc. (mg/ml)	2-oxoglutarate Amount (mg/sample)	Citrate Conc. (mg/ml)	Citrate Amount (mg/sample)	Urine vol. (ml)
Li	0.390 ± 0.021 (48)	6.78 ± 0.049 (24)	0.097 ± 0.011	1.63 ± 0.12	6.14 ± 0.68	2.05 ± 0.10	8.80 ± 0.46	4.5 ± 0.3
Na		6.88 ± 0.063 (24)	0.069 ± 0.008	0.73 ± 0.063	2.60 ± 0.17	1.89 ± 0.10	7.08 ± 0.27	4.3 ± 0.2
Cs	0.272 ± 0.012 (40)	6.84 ± 0.047 (20)	0.100 ± 0.013	0.09 ± 0.011	0.34 ± 0.031	0.24 ± 0.03	0.95 ± 0.14	4.7 ± 0.4
Rb	0.500 ± 0.017 (40)	6.72 ± 0.032 (20)	0.136 ± 0.013	0.096 ± 0.009	0.37 ± 0.034	0.27 ± 0.04	1.11 ± 0.14	4.7 ± 0.20

Sodium chloride was administered at 0.33 mEq/day for 17 days to six animals. Lithium chloride at 0.35 mEq/day for 24 days to six animals. Rubidium chloride at 0.41 mEq/day for 31 days to five animals. Caesium chloride at 0.46 mEq/day for 45 days to five animals. Values are given with their s.e. mean. At this stage the excretion of the administered ion appeared constant and each animal was studied for six consecutive days. Where appropriate results are per 3 hours.

Table 4 Effect of chronic lithium chloride compared to sodium chloride administration on the excretion of 2-oxoglutarate and citrate.

	Urine pH		Urine volume		2-Oxoglutarate Concentration (μmol/ml)		2-Oxoglutarate Total 6 h output (μmol)		Citrate Concentration (μmol/ml)		Citrate Total 6 h output (μmol)	
	Na	Li	Na	Li	Na	Li	Na	Li	Na	Li	Na	Li
Rat 1	6.51 ±0.17	6.47 ±0.11	3.82 ±1.41	6.80 ±1.80	0.317 ±0.076	0.745 ±0.19	1.31 ±0.41	5.07 ±0.65	0.88 ±0.06	1.03 ±0.46	3.77 ±1.16	7.00 ±2.54
Rat 2	6.29 ±0.31	6.31 ±0.21	4.61 ±1.38	9.26 ±0.94	0.304 ±0.15	0.718 ±0.17	1.52 ±0.75	6.80 ±1.70	1.24 ±0.66	1.55 ±0.42	6.09 ±3.80	14.70 ±4.06
Rat 3	6.24 ±0.16	6.36 ±0.11	3.92 ±1.62	8.06 ±1.11	0.18 ±0.12	0.432 ±0.092	0.81 ±0.41	3.76 ±0.83	0.75 ±0.41	1.15 ±0.22	3.37 ±1.86	9.45 ±1.62
Rat 4	6.44 ±0.19	6.66 ±0.16	5.14 ±2.06	12.84 ±1.67	0.216 ±0.26	0.518 ±0.051	1.48 ±1.17	7.00 ±1.44	1.16 ±0.70	1.01 ±0.16	5.95 ±4.94	13.4 ±1.35
Average values	6.37 ±0.23	6.45 ±0.20	4.35 ±1.55	9.24 ±2.66	0.269 ±0.13	0.603 ±0.19	1.28 ±0.75	5.66 ±1.77	1.01 ±0.51	1.18 ±0.38	4.79 ±3.28	11.1 ±4.0
P (one tailed) for difference in average values	N.S.		<0		<0.0005		<0.0005		N.S.		<0.0005	

Sodium chloride was administered in the diet and 6 h collections of urine were made over five days. Lithium chloride (about 3 mEq/kg body weight, daily) was then administered and after 12 days, five successive 6 h urine collections were made. Each value for individual rats is the mean and s.d. of values obtained on five days.

decreases in 2-oxoglutarate and citrate excretion. The urine acidity and volume, following administration of these ions under these conditions, are similar (see Table 3). A more detailed examination (Table 4) of the effect of chronic feeding of lithium was carried out at a higher lithium intake (3 mEq lithium/kg body weight, daily) so that the relative effect of lithium on 2-oxoglutarate and citrate excretion could be observed better. Four rats were given a diet containing sodium chloride (0.6 mEq Na/day) and urine collections were made over 6 h periods on five days. From these collections the pretreatment values (mean and s.d.) of 2-oxoglutarate and citrate excretion, as well as urine pH, were obtained. Lithium (as LiCl in food) was then administered daily at a rate of 3 mEq/kg. Urines (6 h) were collected on five successive days from day 12, when urine pH and organic acid output had stabilized. From these collections, made under identical conditions to the pretreatment samples, the values (mean and s.d.) for the effect of chronic lithium treatment were obtained. In each rat, and in the combined values, lithium produced a significant increase in the excretion of 2-oxoglutarate, both in concentration and in total output over the 6 h period. For citrate there was a significant increase in excretion only for the total 6 h output. In none of the rats was the urine pH significantly changed during the period of collection.

No studies were made of rat blood levels of organic acids following lithium treatment but studies of tissue levels show a statistically significant increase of 2-oxoglutarate and decrease of glutamate following lithium injections. This causes a change in the ratios of glutamate to 2-oxoglutarate, a possible indication of a redox potential change (see Table 5).

Table 6 shows the rate of gluconeogenesis in kidney slices from animals excreting increased amounts of 2-oxoglutarate following (2 h) acute intraperitoneal injections of lithium chloride. Using 10 mM glutamate or succinate as substrates, a small increase due to lithium administration was observed, compared with the controls (animals injected with NaCl solutions). Table 7 shows a comparison of gluconeogenesis, between kidney slices incubated in solutions containing 5 mM lithium chloride and no lithium chloride. The substrate used was glutamate; glucose production and oxygen consumption were measured, neither showing a clear effect of lithium.

Table 5 The effect of a single injection of lithium chloride on levels of 2-oxoglutarate and glutamate in rat kidney.

	Sodium chloride	*Lithium chloride*	*P for comparison of sodium and lithium treatment*
2-Oxoglutarate	0.115 ± 0.0073	0.168 ± 0.018	<0.05
Glutamate	1.82 ± 0.13	1.58 ± 0.055	N.S.
Ratio glutamate: 2-oxoglutarate	15.9 ± 0.98	9.9 ± 1.09	<0.01

Animals (170 g) were given lithium or sodium chloride 0.2 mM by injection (i.p.) and the kidney removed under anaesthesia after 2 hours. Values are expressed as μmol/g wet weight of tissue and represent the mean and s.e. mean of determinations on six animals.

Table 6 The effect of a single injection of lithium chloride on the rate of glucose formation (μmol/g wet weight^{-1} h^{-1}) by slices of rat kidney.

Substrate	*Sodium*	*Lithium*	*P for comparison of lithium and sodium*
Glutamate 10 mM	14.4 ± 0.48	16.7 ± 0.75	<0.05
Succinate	23.6 ± 1.17	27.6 ± 1.29	<0.05

Values represent the mean (and s.e. mean) of three determinations on each of four animals. Animals received a single intraperitoneal injection of either lithium or sodium chloride (0.2 mM) and were killed after 2 hours.

Table 7 Effect of lithium added to the incubation medium on renal gluconeogenesis and oxygen uptake with glutamate (10 mM) as substrate.

	Control	*Lithium 5 mM*
Glucose formation (μmol/g tissue)	18.5 ± 0.7	17.5 ± 0.26
Oxygen uptake (μmol/g tissue)	155 ± 10.8	148 ± 6.1

Values represent the means of six determinations (and s.e. mean) with kidney slices from a single rat.

Discussion

The results show that in the rat, as in man (Bond *et al.*, 1972) administration of lithium ions leads to an increased excretion of 2-oxoglutarate. In the rat, particularly with acute administration (Table 1) this is accompanied by an increased excretion of citrate, a feature not found in man. Chronic studies (Table 4) with the lithium administered in the food would suggest that this difference is minimal. Under these conditions, 2-oxoglutarate output is elevated over 2-fold both in total amount and in concentration. The corresponding changes in citrate are very much smaller, and concentration changes insignificant, while the increase in total amount excreted is little more than half that for 2-oxoglutarate. The fact that lithium ions initially cause an increase of urine bicarbonate and that alkalosis is known to increase organic aciduria (Crawford *et al.*, 1959) could explain some of the changes following acute administration; indeed, the greater increase caused by the citrates of lithium and sodium as compared with their chlorides supports this view. However, with chronic administration of lithium, sodium, rubidium and caesium, the urine is slightly acidic and yet lithium still leads to increased 2-oxoglutarate excretion while caesium and rubidium produce the opposite effect. It seems therefore that a simple alkalotic effect of lithium does not account for the results.

Several reports have suggested that lithium and rubidium may have opposite behavioural effects (Carroll & Sharpe, 1971; Platman, 1971; Fieve, Meltzer, Dunner, Levitt, Mendlewicz & Thomas, 1973) both in the rat and in man. If this is so, then any biochemical explanation of the therapeutic action of lithium would be strengthened by an opposite biochemical effect of rubidium. The effect on organic aciduria is such an instance, and one might predict that caesium, which has a similar effect on the organic acids to rubidium, would induce comparable behavioural changes if some cerebral process occurs which is analogous to the organic aciduria.

The explanation of these effects on organic acid excretion, which could be confined to the kidney, is still obscure. The two main possibilities are an effect on re-uptake in the kidney tubule and an action on metabolism in the kidney, perhaps an inhibition or stimulation of some enzyme. The results are equally consistent with both. The change in tissue levels reported in this study could be secondary to pH changes in acute studies, though chronic studies on tissue levels (at present being carried out in this laboratory) may clarify this point. The small increase in renal gluconeogenesis in slices from rats injected with lithium may also be due to pH changes. However, a lack of an effect on gluconeogenesis does not preclude an effect of lithium on one of the enzymes of glycolysis or of the Krebs cycle. Gluconeogenesis involves the reversal of many, though not all, of these steps, and the fact that different substrates give different rates of glucose production (Krebs, Bennett, DeGasquet, Gascoyne & Yoshida, 1963) unrelated to their proximity to glucose, suggests that transport of the substrate into the cell may be the rate limiting factor. Thus, only a profound effect by lithium on one of the enzymes would be reflected in decreased gluconeogenesis.

Other effects of lithium on carbohydrate metabolism have been reviewed by Mellerup, Plenge & Rafaelson (1973) and Pearson & Jenner (1971). They include actions on hexokinase and pyruvate kinase (Balan, Cernatescu, Trandfirescu & Ababei, 1970) as well as increased glucose uptake and glycogen synthesis in muscle, brain and adipose tissue. There seems little connection between the present findings and these other reported effects on carbohydrate metabolism. It cannot be said that the changes in organic acid excretion are any more relevant in explaining the therapeutic action of lithium, but the demonstration of opposite biochemical changes induced by chronic administration of lithium and rubidium is unique and encouraging. The present study suggests that the effect of lithium on the metabolism and transport of organic acids in tissues (especially brain) could be a fruitful field to investigate.

L. Grant, P. Hopkinson, S. Hill, G. Jennings and P. Wear–the technical staff could have appropriately been joint authors but decided not, even though they did the work and much of the thinking. We thank them as well as Mrs B. Isles and Mrs Y. Barrett for clerical help.

References

BALAN, G., CERNATESCU, D., TRANDFIRESCU, M. & ABABEI, L. (1970). The influence of lithium ions on the activity of hexokinase and pyruvate kinase. *Colleg. Int. Neuropsychopharm. VIIth Int. Cong.*

BERGMEYER, H.-U. & BERNT, E. (1963). In: *Methods of Enzymatic Analysis* ed. Bergmeyer, H.-U. Verlag Chemie GMBH., Weinheim.

BEUTLER, E. & YEH, M.K.Y. (1959). A simplified method for the determination of citric acid. *J. Lab. Clin. Med.*, **54**, 125-131.

BOND, P.A., JENNER, F.A., LEE, C.R., LENTON, E., POLLITT, R.J. & SAMPSON, G.A. (1972). The effect of lithium salts on the urinary excretion of α-oxoglutarate in man. *Br. J. Pharmac.*, **46**, 116-123.

CARROLL, B.J. & SHARP, P.T. (1971). Rubidium and lithium: opposite effects on amine-mediated excitement. *Science*, **172**, 1355-1375.

CRAWFORD, M.A., MILNE, M.D. & SCRIBNER, B.H. (1959). The effects of changes in acid-base balance on urinary citrate in the rat. *J. Physiol., Lond.*, **149**, 413-423.

FIEVE, R.R., MELTZER, H., DUNNER, D.L., LEVITT, M., MENDLEWICZ, J. & THOMAS, A. (1973). Rubidium: biochemical, behavioural and metabolic studies in humans. *Am. J. Psychiat.*, **130**, 55-61.

FISHER, R.A. (1954). *Statistical methods for research workers* (12th ed.). Oliver & Boyd, Edinburgh.

HESS, B. (1963). In: *Methods of Enzymatic Analysis* ed. Bergmeyer, H.-U. Verlag Chemie GMBH, Weinheim.

HOWELLS, G.R., WRIGHT, C.F. & HARRISON, G.E. (1964). A new metabolic cage for rats. *J. Animal Technicians Assoc.*, **14**, 137-140.

JENNER, F.A. (1973). Biochemical studies on the effect of lithium salts: excretion of tricarboxylic acid-cycle intermediary metabolites, inhibition of vasopressin and distribution of other ions in the body. *Trans. Biochem. Soc.*, **1**, 33-38.

KREBS, H.A., BENNETT, D.A.H., DeGASQUET, P., GASCOYNE, T. & YOSHIDA, T. (1963). Renal gluconeogenesis. The effect of diet on the gluconeogenesis capacity of rat kidney cortex slices. *Biochem. J.*, **86**, 22-27.

MELLERUP, E.T., PLENGE, P. & RAFAELSON, O.J. (1973). Lithium ions and insulin: effects on carbohydrate and electrolyte metabolism. *Trans. Biochem. Soc.*, **1**, 54-56.

PEARSON, I.B. & JENNER, F.A. (1971). Lithium in psychiatry. *Nature, New Biol.*, **232**, 532-533.

PITTS, R.F. (1968). *Physiology of the kidney and body fluids.* 2nd ed. Year Book, Chicago: Medical Publishers Inc.

PLATMAN, S.R. (1971). Lithium and rubidium: a role in the effective disorders. *Dis. Nerv. Syst.*, **32**, 604-606.

SCHOU, M. (1968). Lithium in psychiatric therapy and prophylaxis. *J. Psychiat. Res.*, **6**, 67-95.

UMBREIT, W.W., BURRIS, R.H. & STAUFFER, J.F. (1957). *Manometric Techniques.* Burgess Publishing Co., Minneapolis.

(Received August 13, 1973)