Rats given Phenytoin appeared to give a greater than normal 24 hr incorporation of the tritiated dose ($P < 0.2$) while those on ethanol incorporated much less tritium ($P < 0.01$). In the phenytoin treated there was no clear difference from the normal in the type folates synthesized the predominant form being $5CH_3H_4PteGlu_5$ though substantial amounts of tetra, hexa and hepta were also observed (Fig. 1). Two additional animals receiving 2 mg phenytoin showed the same pattern.

In the ethanol treated animals, the monoglutamate substituents were as normal but polyglutamate synthesis appeared to be impaired (Fig. 2). There may be a relationship between the lower level of tritium incorporation in these animals and their reduced capacity for producing polyglutamate folates. In similar studies with methotrexate (L,*N*-[4-{[2,4 diamino-6-pteridinyl) methyl, methyl]-amino}benzoyl]glutamic acid) treated animals tritium incorporation into liver folates was very effectively reduced and the small percentage of label remaining in the liver at 24 hr was normally distributed among polyglutamates with no remaining exogenous PteGlu.[12] These latter results suggested that biosynthesis of polyglutamate derivatives of folate may be necessary to retain exogenous monoglutamate forms within the cells. The ethanol treated animals employed in this study were not at the stage where structural changes in the hepatic tissue were evident to light microscopy, thus ethanol appears to directly affect the biosynthesis of liver pteroylpolyglutamates. Accordingly, this finding suggests a further mechanism for the well known association of folate deficiency and chronic alcoholism,[5] namely that alcohol in preventing the formation of pteroylpolyglutamates inhibits the storage of folate in the body. This substantiates previous work[13] which demonstrated that folate replete alcoholics when placed on a folate deficient diet with ethanol became folate deficient and developed megaloblastic haemopoiesis more rapidly than when they took a similar diet without ethanol.

Departments of Biochemistry and Clinical Medicine
Trinity College,
Dublin 2,
Irish Republic

J. P. BROWN
G. E. DAVIDSON
J. M. SCOTT
D. G. WEIR

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Effect of acute and chronic administration of lithium on steady-state levels of mouse brain choline and acetylcholine*

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THE THERAPEUTIC efficacy of lithium in the treatment of the manic phase of manic-depressive psychosis is well established.[1,2] In addition, lithium prophylaxis may be beneficial in preventing recurrent

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depressions associated with bipolar affective disorders.[3,4] While the mechanism of action of this agent remains an enigma, some attention has been directed to the effect of lithium on steady-state levels and turnover rates of central nervous system neurotransmitter candidates. The putative neurotransmitters which have been investigated most thoroughly include the catecholamines, norepinephrine and dopamine, and the indoleamine, serotonin.[5–10] Since the cholinergic component of the central nervous system has not been investigated as thoroughly, it was of interest to examine the effect of lithium administration on steady-state levels of brain acetylcholine (ACh) as well as one of its precursors, choline.

Male albino CF-1 mice (Carworth Farms, Becton, N.Y.), weighing 14–25 g, were used throughout and allowed free access to food and water. Control and experimental groups were weight-matched. Isotonic lithium chloride was administered intraperitoneally according to two dosage regimes: (1) animals received two injections of 5 mEq/kg of lithium 4 hr apart and were sacrificed 2 hr after the second dose (acute schedule); or (2) animals were treated once daily (a.m.) for 6 days with either 1 or 2 mEq/kg of lithium and sacrificed 90 min after the last injection (chronic schedule). Controls consisted of animals that received equivalent amounts of isotonic sodium chloride or animals that were sham-injected. Control and experimental groups were treated concurrently in order to avoid the possible influence of well-known daily variations in brain ACh[11,12] on experimental results.

All mice were sacrificed by cervical dislocation and immediately taken into a cold-room (4°) where they were decapitated; the brain was removed and one-half the whole brain (minus pons-medulla and cerebellum) weighed and homogenized in 1 ml of 1 N formic acid–acetone (15:85, v/v) according to the procedure of Toru and Aprison.[11] Time between death and homogenization was about 60 sec. After a 600 $g \times 10$ min centrifugation, 25-μl aliquots of the supernatant were analyzed for ACh and choline. ACh was assayed by the radio-enzymatic method of Goldberg and McCaman.[13] Choline was assayed by a minor modification of the ACh method.[14] All assays were performed in duplicate. Data were analyzed by Student's t-test.[15]

Acute administration of large doses of lithium produced a small, but statistically significant ($P < 0.05$), 17 per cent decrease in whole brain ACh when compared to sodium controls (Table 1). Brain choline levels were not affected by acute treatment with lithium (Table 1). Chronic administration of lithium at doses of 1 or 2 mEq/kg/day for 6 days produced no significant change in steady-state levels of brain choline or ACh (Table 2).

The present experiments indicate that lithium administration in low doses does not alter steady-state levels of mouse brain choline or ACh. The relatively small decrease in brain ACh apparent after acute administration of large doses of lithium suggests that the ability of this ion to interfere with the storage and/or metabolism of ACh, *in vivo*, is minimal. Similarly, Bowers and Rozitis[16] have shown that electrically stimulated release of ACh from cortical slices prepared from rats chronically treated with lithium did not differ from sodium-treated controls. However, the lack of effect of lithium on steady-state levels of choline and ACh does not preclude an effect on the turnover of either of these compounds.

TABLE 1. ACUTE ADMINISTRATION OF LITHIUM ON STEADY-STATE LEVELS OF MOUSE BRAIN CHOLINE AND ACETYLCHOLINE*

Treatment	N†	Acetylcholine (nmoles/g wet wt)	P‡	N	Choline (nmoles/g wet wt)	P‡
Control§	5	18·6 ± 1·46‖		5	50·8 ± 1·82	
			> 0·05			> 0·05
NaCl	5	17·9 ± 0·80		5	56·3 ± 2·27	
			< 0·05			> 0·05
LiCl	5	15·4 ± 0·60		4	61·2 ± 2·56¶	

* Mice were treated with 5 mEq/kg of either LiCl or NaCl, i.p., followed 4 hr later by a second injection of 5 mEq/kg. Two hr after the second injection the animals were sacrificed and the brain was analyzed for ACh and choline.

† Number of animals.

‡ Level of significance comparing controls to sodium and sodium to lithium.

§ Sham-injected controls.

‖ Values are means ± S. E. M.

¶ $P < 0.05$ comparing sham controls to lithium-treated.

TABLE 2. CHRONIC ADMINISTRATION OF LITHIUM ON STEADY-STATE LEVELS OF MOUSE BRAIN CHOLINE AND ACETYLCHOLINE*

Treatment	N†	Acetylcholine (nmoles/g wet wt)	N	Choline (nmoles/g wet wt)
Control‡	10	21·5 ± 0·52§	5	67·3 ± 2·97
NaCl (1)‖	9	20·4 ± 0·85	5	73·9 ± 4·60
NaCl (2)	7	22·0 ± 0·85	2	64·0 + 64·2¶
LiCl (1)	10	19·6 ± 1·51	5	71·3 ± 3·80
LiCl (2)	10	19·7 ± 1·18	5	66·8 ± 2·67

* Mice were treated daily with 1 or 2 mEq/kg of either sodium chloride or lithium chloride for 6 days. Ninety min after the last injection animals were sacrificed and the brain was analyzed for choline or ACh. There were no significant differences between lithium groups and their corresponding sodium controls or between sodium and sham controls.

† Number of animals.

‡ Sham-injected controls.

§ Values are means ± S. E. M.

‖ Numbers in parentheses refer to doses of either 1 or 2 mEq/kg/day.

¶ Represents individual numbers.

Johns Hopkins School of Hygiene,
Department of Environmental Medicine,
Baltimore, Md. 21205, *U.S.A.*

ROBERT D. KRELL*
ALAN M. GOLDBERG

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* Present address: Smith Kline & French Laboratories, Department of Pharmacology, 1500 Spring Garden St., Philadelphia, Pa. 19101.