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Effect of lithium administration on neural enzymes in rats

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Several investigations have been carried out [1–3] to determine the specificity of lithium-therapy for manic depressive psychosis. There are reports [4, 5] that lithium affects thyroid function and administration of lithium salts to animals brings about the hypothyroid condition [6]. Bera and Chatterjee [7] demonstrated that altered thyroid condition could bring about alteration in the release of the neural lysosomal enzymes and could also diminish the levels of some hydrolases in young hypothyroid rats. In this communication the action of lithium salt on the activities of a few enzymes in different subcellular fractions isolated from neural tissues in rats has been studied.

Male albino rats weighing 90–100 g were divided into two groups of 6 rats each. The first group was given a basal diet [8] only, while the second group of animals was fed basal diet along with an oral feeding (by stomach tube) of lithium as lithium carbonate (suspension in distilled water) at a dose of 2 meq/kg body wt for three weeks. Animals of the first group were pair-fed with respect to the animals of the second group. After the treatment the animals were sacrificed, the serum was collected for the estimation of lithium by flame photometry using Speektromon-381L and the brain tissues were taken out for biochemical investigations. The tissue homogenates were subfractionated as described previously [7]. Activities of acid phosphatase and alkaline phosphatase were assayed according to the method of Michell [9], activities of arylsulphatase and cholinesterase were assayed following the method of Farooqui *et al.* [10] and of Augustinsson [11], respectively. Protein content of the enzyme preparation was determined by the method of Lowry *et al.* [12] and that of the homogenate fraction was determined by the method of Gornall *et al.* [13].

Food consumption by the lithium-treated animals was lower only for the 4–6 days in comparison to that of normal control. It was observed that administration of lithium initially caused a marked retardation in the growth rate after which body weights recovered to an appreciable extent. This finding is in agreement with the results obtained by other investigators [14]. Serum lithium concentration was found to be approximately 1.0–1.8 meq/l. in the case of lithium-treated rats.

Lithium is known to influence brain functions [15, 16] and in respect of enzyme activity in neural tissues. Abreu and Abreu [17–19] reported that in the case of succinic dehydrogenase and fumarase, the specific activities were increased while the activity of aconitase was inhibited by lithium treatment. It has been noted here that there was no alteration in the activity of alkaline phosphatase, but the activities of acid phosphatase (Table 1) and arylsulphatase (Table 2) in neural tissue were found to be decreased in the various subcellular fractions including the homogenate fraction. The total activity obtained by disrupting the synaptosomal fraction by the addition of 0.1% (v/v)

Table 1. Effect of lithium administration on the activity of neural acid phosphatase in different organelles of rats

Group	Total weight gain (g/100 g body wt)	Sp. act. (μg of *p*-nitrophenol liberated/min/mg of protein)				
		Homogenate	Mitochondrial fraction	Synaptosomal fraction	Microsomal fraction	Supernatant fraction
1. Basal diet fed	33.6 ± 3.02	2.16 ± 0.37	3.06 ± 0.32	5.56 ± 0.54 (8.32 ± 0.66)	3.35 ± 0.34	2.07 ± 0.16
2. Basal diet fed + Li^+ as Li_2CO_3	31.1 ± 2.33	1.74 ± 0.26*	2.84 ± 0.25	4.23 ± 0.45‡ (6.55 ± 0.38)‡	2.77 ± 0.30†	1.63 ± 0.40*

Group 2 has been compared with group 1.
The data inside the parenthesis denotes the total activity of the enzyme.
*† Values significantly different from control ($P < 0.05$ and 0.02, respectively).
‡ Mean value highly significant ($t > 4.59$ for 10 degrees of freedom).
Each result is expressed as mean ± S.E.M. of six experiments; each on a different animal.

Table 2. Effect of lithium administration on the activity of neural arylsulphatase in different organelles of rats

Group	Sp. act. (Unit* of *p*-nitrocatechol/hr/mg of protein)				
	Homogenate	Mitochondrial fraction	Synaptosomal fraction	Microsomal fraction	Supernatant fraction
1. Basal diet fed	9.0 ± 1.15	34.7 ± 3.88	46.0 ± 4.44 (72.5 ± 5.96)	11.3 ± 1.59	9.1 ± 1.27
2. Basal diet fed + Li^+ as Li_2CO_3	7.5 ± 0.83†	29.6 ± 5.00	36.4 ± 3.14§ (60.4 ± 6.83)‡	8.3 ± 1.36‡	6.1 ± 1.42‡

Group 2 has been compared with group 1.
The data inside the parenthesis denotes the total activity of the enzyme.
* Unit = 5 Klett reading.
†‡ Mean values significantly different from control ($P < 0.05$ and 0.01, respectively).
§ Mean value highly significant ($t > 4.59$ for 10 degrees of freedom).
Each result is expressed as mean ± S.E.M. of six experiments; each on a different animal.

Triton-X-100, was also found to decrease. A decrease in the activities of these enzymes in the microsomal fractions with simultaneous inhibition in both the free and total activities in the synaptosomal fraction indicated a diminished level of the net amount of these enzymes under lithium-administered condition.

In vitro study indicates that lithium carbonate at 10 mM concentration could inhibit the activity of acid phosphatase to the extent of approximate 38 per cent and this inhibition was slightly lowered (from 38 per cent inhibition it is reduced to 25 per cent) by dialysis of the enzyme against 0.02 M acetate buffer (pH 5.0) containing 1 mM EDTA.

In the case of cholinesterase there was no alteration in the activity under the influence of lithium, which was also previously observed by other investigators [20].

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REFERENCES

1. H. Corrodi, K. Fuxe and M. Schou, *Life Sci.* **8**, 643 (1969).
2. J. J. Schildkraut, S. M. Schanberg and I. J. Kopin, *Life Sci.* **5**, 1479 (1966).
3. H. Komiskey and C. K. Buckner, *Neuropharmacology* **13**, 159 (1974).
4. J. H. Lazarus and E. H. Bennie, *Acta endocr.* **70**, 266 (1972).
5. (Ed. D. Glick) p. 43. Interscience, New York (1957). *Life Sci.* **7**, 1257 (1968).
6. P. T. Mannisto, J. Leppaluoto and P. Virkkunen, *Acta endocr.* **74**, 492 (1973).
7. H. Bera and G. C. Chatterjee, *J. Neurochem.* **24**, 859 (1975).
8. G. C. Chatterjee, R. K. Roy, S. K. Banerjee and P. K. Majumder, *J. Nutr.* **103**, 509 (1973).
9. R. H. Michell, M. J. Karnovsky and M. L. Karnovsky, *Biochem. J.* **116**, 207 (1970).
10. A. A. Farooqui and B. K. Bachhawat, *Biochem. J.* **126**, 1025 (1972).
11. K. Augustinsson, *Methods of Biochemical Analysis.* Vol. 5. (Ed. D. Glick) p. 43. Interscience, New York (1957).
12. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
13. A. G. Gornall, C. J. Bardawill and M. M. David, *J. biol. Chem.* **177**, 751 (1949).
14. P. A. Bond, B. A. Brooks and A. Judd, *Br. J. Pharmac.* **53**, 235 (1975).
15. P. Poitou, F. Guerinot and C. Bohuon, *Psychopharmacologia* **38**, 75 (1974).
16. N. A. Peterson and E. Raghupathy, *Biochem. Pharmac.* **23**, 2491 (1974).
17. L. A. Abreu and R. P. Abreu, *Nature, New Biol.* **236**, 254 (1972).
18. L. A. Abreu and R. P. Abreu, *Experientia* **29**, 446 (1973).
19. L. A. Abreu and R. P. Abreu, *Experientia* **30**, 1056 (1974).
20. L. L. Simpson, *Psychopharmacologia* **38**, 145 (1974).

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Activation of nitrofurantoin to a mutagen by rat liver nitroreductase

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Many nitrofurans have been found to be mutagenic [1, 4, H. S. Rosenkranz, unpublished results] and to be endowed with oncogenic potential as well [5, 6]. Nitrofurantoin (1-[(5-nitrofurylidene)amino]-hydantoin, furantoin), the nitrofuran utilized most in antimicrobial chemotherapy is not carcinogenic [5, 6], yet it possesses mutagenic and DNA-modifying activity when tested in bacterial systems [2, 3 and H. S. Rosenkranz, unpublished results]. Because there has been a good correlation between mutagenicity in bacterial systems and carcinogenicity in animals [7–9], nitrofurantoin occupies a unique position in chemical carcinogenesis. In view of this situation and its probable relevance to the relationship between mutagens and carcinogens, we have investigated the mutagenicity of nitrofurantoin.

The Salmonella mutagenicity assay developed by Ames *et al.* [10] was used in this study. The indicator micro-organism (histidine auxotrophs), the test agent and when indicated the rat liver preparation (including the required co-factors) [7] were incorporated into the overlay. These mixtures were incubated at 37° in the dark [11] for 46 hr and revertants (mutants) to histidine-independence were enumerated. When anaerobic conditions were required, the plates were placed in a Gas-Pak system (BBL, Cockeysville, Md.), and incubated at 37° for 16 hr, whereupon they were incubated an additional 30 hr aerobically. Liver microsomes (actually the S-9 post-mitochondrial fraction) were prepared from Sprague–Dawley rats by a previously described procedure [7].

The indicator micro-organism was *Salmonella typhimurim* TA100 [12] and a mutant (TA100-FR1) derived therefrom which was deficient in nitro-reductase. TA100-FR1 was obtained by selection [13] of a strain capable of growth in the presence of nitrofurazone (20 μg/ml, 5-nitro-2-furaldehyde semicarbazone). The absence of nitroreductase (4 per cent of control) was confirmed colorimetrically [13]. TA100-FR1 was resistant to the growth inhibitory properties of other nitrofurans, nitro-heterocyclics and nitro-aromatic compounds which indicates that it is deficient in a non-specific nitroreductase.

It might be argued that the mutagenicity of nitrofurantoin in bacterial cells derives from its conversion by prokaryotes to an active hydroxylamino derivative but that this enzymic activity is lacking in mammals which could account for its lack of carcinogenicity if it be assumed [7] that the active mutagenic intermediate is also the metabolite responsible for the carcinogenic event. To test this possibility we have prepared a microbial indicator strain (TA100-FR1) deficient in nitroreductase. Unlike its parent (TA100), this strain is not mutagenized by nitrofurantoin (Table 1), although it does respond normally to a series of mutagens lacking the nitro-function (Table 1). Upon addition of a preparation derived from rat liver, the mutagenic activity of nitrofurantoin for TA100-FR1 was restored (Table 1). When the mixtures were incubated anaerobically for several hr, nitrofurantoin was mutagenic for TA100-FR1 even in the absence of the liver enzymes (Table 1); this appears to be due to the presence in these bacteria of a second, oxygen-labile, non-specific nitroreductase (unpublished results). However, upon supplementation with