Biochemical Effects Due to Interaction of Lithium Ions and Disulfiram in Rats (34072)

S. A. MILLARD, A. E. ROGGEVEEN, AND S. J. GEYER
(Introduced by E. M. Gál)

Neurochemical Research Division, Department of Psychiatry, College of Medicine, University of Iowa, Iowa City, Iowa 52240

Biological manifestations of drug incompatibility are not uncommon. Monoamine oxidase inhibitors and tricyclic antidepressants, phenothiazines and barbiturates, and barbiturates and alcohol are well-known examples. The recent adoption of lithium carbonate as a treatment for manic-depressive psychosis (1) and its elevation from experimental to routine therapy in this disorder should evoke an interest in the possible effects of administration of other drugs to patients on lithium carbonate therapy. One such combination would be lithium therapy and administation of disulfiram (tetraethylthiuram disulfide, Antabuse) in manic depressive alcoholics. Disulfiram, in addition to its inhibition of aldehyde dehydrogenase (2) and dopamine-β-oxidase (3) has been shown to be a potent inhibitor of cerebral hexokinase (4) and has been implicated in inhibition of glucose metabolism at a level below hexokinase, although the exact localization of this inhibition was not determined (5). Lithium ion has also been shown to increase *in vitro* glucose uptake by rat diaphragm and epididymal fat, while *in vivo* LiCl produced prolonged and acute hypoglycemia (6). In light of these biochemical effects, an interaction between lithium salts and disulfiram affecting cerebral metabolism of biogenic amines was explored by testing the various combinations of these drugs in rats.

Materials and Methods. Male hooded rats, 250–350 g, were used. Drugs were administered intraperitoneally (ip) according to the following protocol, and the animals sacrificed 24 hr after the last injection: a) "Lithium" - 0.6 *M* LiCl, 5 mEq/kg for 4 days; b) "Disulfiram" - as a suspension in 0.3% tragacanth gum, 60 mg/kg, for 2 days; c) "Propylene glycol" - 1 ml *asym.*-propylene glycol, 3.5 g/kg, for 2 days; d) "Disulfiram-lithium" - disulfiram as in (b) for 2 days, followed by LiCl as in (a) for 4 days; e) "Lithium-disulfiram" - LiCl for 4 days with disulfiram simultaneously on the last 2 days; f) "Lithium-propylene glycol" - LiCl as in (a) for 4 days with propylene glycol as in (c) simultaneously on the last 2 days; g) "Lithium-tragacanth vehicle" - LiCl as in (a) for 4 days with 1 ml 0.3% tragacanth gum for 2 days.

The two different sequences of injection of lithium chloride and disulfiram where chosen to simulate the clinical drug scheduling of a patient being treated with either drug prior to administration of the second.

Serum Li^+ concentrations were determined from blood removed from the jugular vein of rats under light ether anesthesia. Serum was deproteinized with 20% trichloroacetic acid and Li^+ was measured on a Beckman DU spectrophotometer with flame photometer attachment. Brain and muscle (cranial portion of the platysma) were analyzed for Li^+ as described by Schou (7).

Muscle and serum lactate was determined by the *p*-hydroxydiphenyl method (8). Serum was removed from the jugular vein as described above and deproteinized with 10% trichloroacetic acid. Four hours later, animals were decapitated and about 500 mg of biceps femoris rapidly removed and homogenized in 5 ml of cold 10% trichloroacetic acid. Aliquots of 1 ml were taken for assay. All assays were run in duplicate.

Animals were decapitated and brains were removed immediately in a cold room at 4°.

The brains were cut into symmetrical halves and one half assayed for tryptophan-5-hydroxylase activity (9). The other half was assayed for 5-HT and NE (10). The method is rapid, and for NE gives results in accord with those obtained with other similar procedures (9, 11). However, the determination of 5-HT is somewhat problematic.[1] In this study we obtained a value of 0.86 μg/g 5-HT using the simultaneous extraction of 5-HT and NE. (This value is probably high due to incomplete extraction of interfering fluorophores and results for 5-HT must be considered relative to control values rather than as an indication of absolute 5-HT levels.)

The *in vitro* effect of disulfiram on purified rat lactate dehydrogenase as assayed spectrophotometrically from the lactate side (14).

Results and Discussion. The combination of lithium salt and disulfiram resulted in fatalities only when the animals were treated with disulfiram prior to lithium chloride injections. As can be seen in Table I, mortality in these rats can be correlated with a substantial weight loss, with the succumbing animals having an average weight loss nearly twice that of the survivors on the same drug schedule. In addition, it was found that some of the disufiram–lithium animals showed anomalous serum, brain, and muscle lithium values, almost ten times that for the rest of the same group (which were not different from controls, see Table II). Of the 11 animals with excessive serum lithium concentration, 9 had suffered weight losses of more than 40 g, and 5 of them had lost more than 60 g. There are reports of increased urine volume after lithium salt administration or lithium intoxication in animals as well as oliguria in terminal stages of lithium poisoning (15). Therefore, urine excretion and

TABLE I. Mortality and Weight Loss in Rats Receiving Lithium Chloride and Disulfiram.

Condition[a]	Mortality, no. of deaths/no. of animals	Average weight change (g)
Control	0/6	+ 7
Disulfiram	0/12	—13
Lithium	0/24	—10
Disulfiram-lithium	9/40	—38 (S)[b] —63 (N)
Lithium-disulfiram	0/30	—15
Lithium-propylene glycol	0/22	—22
Propylene glycol	0/6	— 5

[a] Dosage and injection schedule as in Methods. Animals were observed up to 24 hr after last injection.

[b] (S) = survivors; (N) = nonsurvivors.

[1] The original description of this extraction procedure (10) gives whole brain 5-HT values for rat of 0.55 μg/g, which may be compared to values of 0.48-0.50 μg/g by other methods (*e.g.*, 11, 12). Yet a second report by the same authors (13) using this procedure lists values of 0.76-0.89 μg/g for rat brain, with no comment on the reasons for the discrepancy from the previous values.

TABLE II. Serum, Muscle, and Brain Li^+ in Rats Receiving Lithium Chloride, Disulfiram, and Propylene Glycol.

Condition[a]	Serum Li^+ (mEq/l ± SD)	Muscle Li^+ (mEq/kg ± SD)	Brain Li^+ (mEq/kg ± SD)
Lithium	0.27 ± 0.14 (22)[b]	0.42 ± 0.38 (12)	0.72 ± 0.39 (12)
Disulfiram-lithium	0.29 ± 0.09 (17)	0.31 ± 0.21 (14)	0.83 ± 0.44 (14)
and	2.53 ± 1.42 (11)*	2.54 ± 1.22 (4)*	3.80 ± 3.00 (4)[c]
Lithium-disulfiram	0.29 ± 0.14 (20)	0.34 ± 0.29 (10)	1.39 ± 1.27 (11)
Lithium-propylene glycol	0.24 ± 0.10 (17)	0.46 ± 0.37 (14)	0.89 ± 0.32 (14)
and	2.22 ± 1.11 (5)*	3.77 ± (1)	8.21 ± (1)

[a] Dosage and injection schedule as described in Methods.

[b] Number of animals in parentheses.

[c] Range 1.50–8.11.

* $p < .01$.

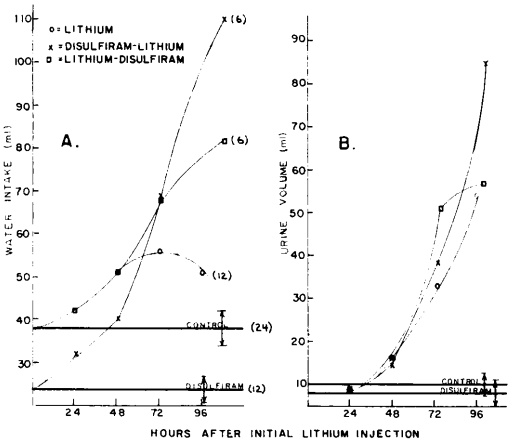

FIG. 1. A. Water consumption and B. Urine excretion after lithium chloride and disulfiram. Number of animals is in parentheses.

water consumption were monitored daily during the course of drug administration. These data are shown graphically in Fig. 1. As might be expected, both water consumption and urine excretion are greater in animals receiving lithium chloride. However, animals receiving combinations of lithium and disulfiram had higher values for both urine excretion and water intake, with disulfiram-lithium animals highest in both cases.

The results of determinations of serum, muscle, and brain Li^+ are summarized in Table II. Additionally, there were no differences in any of these parameters between rats receiving LiCl alone or with the disulfiram vehicle, 0.3% tragacanth gum [protocol (g)]. Serum and brain values are in agreement with those of Corrodi *et al.* (16). As mentioned above, Li^+ values for the disulfiram-lithium group fell into two statistically different groups.

Disulfiram is insoluble in water and some studies have employed *asym.* propylene glycol as an injection vehicle (17, 18). Our preliminary studies indicated that *asym.* propylene glycol caused significant lithium retention and therefore it was not used as a vehicle in this study. Like the disulfiram-lithium rats, these animals could be separated into two groups on the basis of the serum lithium levels. However, in these animals the high serum Li^+ values could not be correlated with excessive weight loss. In a later experiment when brain and muscle Li^+ were also examined, one rat of this group had a high serum Li^+ concentration with a correspondingly high brain and muscle Li^+ (Table II).

Lithium ion is also reported to stimulate lactate production in human erythrocytes, probably mediated by its effect on membrane ATP-ase (20). The animal body metabolizes *asym.* propylene glycol to lactate; consequently the effect on lithium retention might be mediated through lactate. Animals of the disulfiram-lithium group also retained Li^+ at high levels and the possibility that disulfiram might have an effect on systems relating to lactate production in the body was therefore considered. Serum and muscle lactate were examined to see if a correlation could be found between retention and lactate levels. The results in Table III indicate no significant differences in muscle lactate; however, serum lactate levels were significantly elevated in the lithium-propylene glycol and disulfiram–lithium groups. It is difficult to draw a conclusion from these data, since animals with either extreme weight loss or high tissue and serum Li^+ values were indistinguishable from the others in the group with respect to serum lactate. Additionally, *in vitro* disulfiram at 10^{-4} *M* did not inhibit rat lactate dehydrogenase from the lactate side.

TABLE III. Serum and Muscle Lactate in Rats Receiving Lithium Chloride, Disulfiram, and Propylene Glycol.

Condition[a]	Serum lactate (mg/100 ml ± SD)	Muscle lactate (mg/100 g ± SD)
Control (6)[b]	25.7 ± 4	131 ± 30
Lithium (6)	31.7 ± 6	155 ± 20
Disulfiram-lithium (12)	41.0 ± 7*	139 ± 23
Lithium-disulfiram (6)	31.0 ± 6	122 ± 30
Lithium-propylene glycol (10)	39.4 ± 6*	179 ± 66

[a] Dosage and injection schedule as described in Methods.

[b] Numbers of animals in parentheses.

* $p < .01$.

TABLE IV. Cerebral Tryptophan-5-Hydroxylase, 5-HT, and NE in Rats Receiving Lithium and Disulfiram.

Condition[a]	Tryptophan-5-hydroxylase (mμmole /mg/hr ± SD)	5-HT (% of control ± SD)	NE (% of control ± SD)
Control	8.8 ± 3.9 (14)[b]	100[c] ± 5 (6)	100[c] ± 12 (7)
Disulfiram	4.6 ± 1.5 (10)	102 ± 8 (6)	110 ± 12 (6)
Lithium	8.9 ± 2.4 (12)	107 ± 12 (12)	95 ± 17 (11)
Propylene glycol	7.2 ± 2.8 (6)	90 ± 12 (6)	116 ± 11 (6)**
Disulfiram-lithium	7.1 ± 2.8 (10)	96 ± 8 (12)	97 ± 9 (13)
Lithium-disulfiram	8.9 ± 3.1 (10)	103 ± 10 (12)	84 ± 13 (11)*
Lithium-propylene glycol	—	99 ± 12 (6)	90 ± 8 (6)

[a] Dosage and schedules as described in Methods.

[b] Numbers of animals in parentheses.

[c] Control values using combined 5-HT-NE extraction (9) were 0.86 μg/g (5-HT) and 0.56 μg/g (NE) (see Methods).

* $p < .02$; ** $p < .05$.

There was no significant effect on cerebral 5-HT, either as reflected in brain 5-HT levels or in the rate-limiting step of 5-HT biosynthesis, cerebral tryptophan-5-hydroxylation. The intraperitoneal dose of disulfiram used in this study, 60 mg/kg, is only 15% of that required to inhibit dopamine-β-oxidase *in vivo*, 400 mg/kg (3). An examination of Table IV indicates that only when the rats were pretreated with lithium chloride did this dose of disulfiram result in slightly lowered cerebral norepinephrine levels. Animals receiving propylene glycol alone appeared to have slightly elevated norepinephrine.

The exact cause of the potentiation of disulfiram and lithium chloride remains undetermined, as does the reason for lithium retention in animals receiving disulfiram–lithium and lithium–propylene glycol combinations. However, a recent study (21) showed increased retention and a difference in distribution of lithium in acutely manic patients. Clinical improvement in these patients was accompanied by release of stored lithium. It would be of interest to examine lactate levels in such patients.

Summary. Rats pretreated with disulfiram had a mortality rate of 23% when injected with 5 mEq/kg LiCl for 4 days. Some animals receiving lithium chloride in combination with disulfiram or *asym.* propylene glycol retained lithium in their serum and tissues at higher levels than those receiving only lithium chloride. Serum lactate was elevated in animals pretreated with disulfiram or receiving *asym.* propylene glycol and LiCl. No effect was found on brain 5-HT or tryptophan-5-hydroxylase and only slight effects on brain NE.

The authors thank Mrs. A. Kubose, Mrs. U. Follman, and Mr. R. Fetherolf for their technical assistance and Dr. Carl S. Vestling of the Department of Biochemistry, University of Iowa, for the lactate dehydrogenase assays. They are grateful to Dr. John Clancy for pointing out the lack of information in this clinically relevant area of research.

1. Baastrup, P. C. and Schou, M., Arch. Gen. Psychiat. **16**, 162 (1967).

2. Hunter, F. E. and Lowry, O. H., Pharmacol. Rev. **8**, 89 (1956).

3. Musacchio, J., Kopin, I. J., and Snyder, S., Life Sci. **3**, 769 (1964).

4. Strömme, J. H., Biochem. Pharmacol. **12**, 157 (1963).

5. Strömme, J. H., Biochem. Pharmacol. **12**, 705 (1963).

6. Bhattacharya, G., Biochim. Biophys. Acta **93**, 644 (1964).

7. Schou, M., Acta Pharmacol. Toxicol. **15**, 115 (1958).

8. Barker, S. B., Methods Enzymol. **3**, 241 (1957).

9. Gál, E. M., Heater, R. D., and Millard, S. A., Proc. Soc. Exptl. Biol. Med. **128**, 412 (1968).

10. Maickel, R. P., Cox, R. H., Jr., Saillant, J. and Miller, F. P., Int. J. Neuropharmacol. **7**, 275 (1968).

11. Gál, E. M., Drewes, P. A., and Barraclough, C. A., Proc. Intern. Pharmacol. Meeting, 1st, Stockholm **8**, 107 (1962).

12. Gál, E. M. and Millard, S. A., Biochem. Pharmacol. **17**, 2193 (1968).

13. Miller, F. P., Cox, R. H., Jr., and Maickel, R. P., Science **162**, 463 (1968).

14. Anderson S. R., Florini, J. R., and Vestling, C. S., J. Biol. Chem. **239**, 2991 (1964).

15. Schou, M., Pharmacol. Rev. **9**, 17 (1957).

16. Corrodi, H., Fuxe, K., Hökfelt, T., and Schou, M., Psychopharmacologica **11**, 345 (1967).

17. Gál, E. M. and Greenberg, D. M., Proc. Soc. Exptl. Biol. Med. **85**, 252 (1954).

18. Smith, A. A. and Wortes, S. B., Biochim. Biophys. Acta **40**, 569 (1960).

19. Jokivartio, E., Acta Pharmacol. **6**, 107 (1950).

20. Whittam, R., Ager, M. F., and Wiley, J. S., Nature **202**, 1111 (1964).

21. Greenspan, K., Goodwin, F. K., Bunney, W E., and Durell, J., Arch. Gen. Psychiat. **19**, 664 (1968).

Received Feb. 17, 1969. P.S.E.B.M., 1969, Vol. 131.