LEVINE, B. B. (1961). *Archs Biochem. Biophys.*, **93**, 50–55.
SCHNEIDER, C. H. (1970). In: *Penicillin Allergy, Clinical and Immunological Aspects*, pp. 23–58. Editors: Stewart, G. T. & McGovern, J. P. Springfield: C. C. Thomas.
SCHNEIDER, C. H. & DE WECK, A. L. (1968). *Biochim. Biophys. Acta*, **168**, 27–35.
SCHWARTZ, M. A. & WU, G. M. (1966). *J. pharm. Sci.*, **55**, 550–555.
SCHWARTZ, M. A. (1968). *Ibid.*, **57**, 1209–1211.
SCHWARTZ, M. A. (1969a). *Ibid.*, **58**, 643–661.
SCHWARTZ, M. A. (1969b). *J. medl Chem.*, **12**, 36–38.
SCHWARTZ, M. A. & DELDUCE, A. J. (1969), *J. pharm. Sci.*, **58**, 1137–1139.
WAGNER, E. S., DAVIS, W. W. & GORMAN, M. (1969). *J. medl Chem.*, **12**, 483–487.

Lithium and ethanol preference

Recently lithium has been reported to reduce ethanol consumption in chronic alcoholics (Wren, Kline & others, 1974) and it has also been used in the treatment of ethanol withdrawal (Sellers, Copper & Zilm, 1974) but little is known about the interactions between lithium and ethanol. We now present some preliminary data to show that treatment with a dose of lithium, comparable to that used in man reduced significantly the volitional consumption of ethanol in rats. In addition, the concentrations of brain acetylcholine showed good correlation with the behavioral observations.

Adult male Sprague-Dawley rats, 200 to 250 g, were kept in individual cages at a constant 70°F. Two graduated glass drinking tubes (Kimax Instrument Co.) were fitted onto the outside of each cage, one filled with water and the other with varying concentrations of ethanol. The positions of the tubes were changed daily and also different tubes were used so as to prevent the development of a position habit. The ethanol used was diluted from 95% ethanol with tap water (v/v) to the required concentration. The preference-aversion cut off concentrations and the base-line consumption for each rat was determined as described by Amit, Stern & Wise (1970). Food, water and ethanol were freely available, consumption and body weight were recorded at 10 a.m. each day. Lithium (0·3 m equiv kg^{-1}) was given intraperitoneally at 12 hourly intervals as lithium chloride adjusted to an isotonic solution with distilled water. The control animals were treated similarly with normal saline. The concentrations of lithium in the blood and brain were well below the toxic levels, similar doses having previously yielded mean plasma concentrations of less than 0·2 m equiv $litre^{-1}$ and brain concentrations of 0·05 m equiv kg^{-1} approximately. At the concentrations of lithium used, there was no significant effect on water consumption and diuresis was minimal compared with the controls. At the end of the treatment, the rats were killed and the brains were dissected on ice. One side of the brains was used for choline transferase determination; the other side was extracted and assayed for acetylcholine. Choline transferase activity was determined using 1-[^{14}C]acetylcoenzyme A as substrate according to Ho, Singer & Gershon (1971). Acetylcholine was extracted from the brains, after homogenization in a glass homogenizer, with at least 4 volumes of 10% trichloroacetic acid, and then extracted with ether as described by Hebb (1963). The extracts were assayed biologically using the guinea-pig isolated ileum preparation as described by Bentley & Shaw (1952).

Within the same strain of rats, there were significant individual variations in the preference-aversion cut off concentrations; among 30 rats, these ranged from 3 to 11%. Rats given lithium showed a marked reduction in their daily consumptions of ethanol irrespective of the cut-off concentrations used (Fig. 1). The total fluid intake appeared not to have been altered significantly as the decrease in ethanol intake was compensated by an increase in water consumption. The reduction of ethanol consumption lasted throughout the whole treatment period, but returned to the base-line 2 days after the discontinuation of lithium.

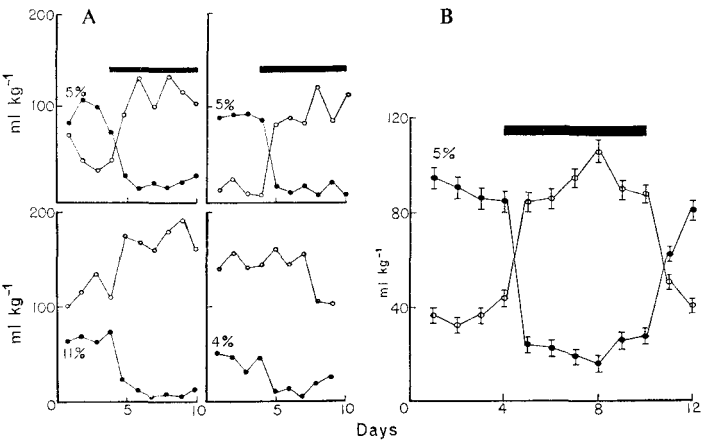

FIG. 1A. The effects of lithium treatment for period shown by bars (13 mg kg^{-1}, i.p., twice daily on ethanol selections in individual rats. Preference-aversion cut-off concentrations as indicated were used for the estimation. Daily consumptions of water (○) and ethanol (●) (4 to 11%) were measured and expressed as ml kg^{-1}.

B. The duration of reduction in ethanol preference and the recovery after the discontinuation of lithium treatment. A group of 8 rats was used and the concentration of ethanol was 5% (v/v). The parameters are the same as in Fig. 1.

The effects of lithium on the cholinergic system were examined in view of our recent observations that ethanol preference may be related to central cholinergic activities (Ho & Kissin, 1974). Results obtained showed that the concentrations of brain acetylcholine were significantly ($P < 0{\cdot}001$) lowered at 1, 2 and 4 days of lithium treatment. Mean values obtained for brain acetylcholine in the control groups were $2{\cdot}23 \pm 0{\cdot}25$ $\mu g\ g^{-1}$ ($n = 10$) compared with $1{\cdot}51 \pm 0{\cdot}12$ $\mu g\ g^{-1}$ ($n = 5$), $1{\cdot}66 \pm 0{\cdot}22$ $\mu g\ g^{-1}$ ($n = 5$) and $1{\cdot}57 \pm 0{\cdot}09$ $\mu g\ g^{-1}$ ($n = 5$) at 1, 2 and 4 days of lithium treatment respectively. There was no significant difference in the activities of brain choline transferase. These findings appeared to be in good agreement with suggestions we made previously (Ho & Kissin, 1974) that ethanol preference may be related to central cholinergic activities since the brain content of acetylcholine in a specific genetic strain of high ethanol preference mice, $C_{57}B1/6J$, is significantly higher than a low ethanol preference strain, DBA/2J mice. Lithium treatment (13 mg kg^{-1}) in the C57B1 mice was also related to a significant reduction in consumption using a 5% solution of ethanol under a free choice situation ($P < 0{\cdot}001$). Other possibilities exist, for example, lithium treatment may affect the uptake and release of acetylcholine. Lithium decreases the release of acetylcholine from the cortex of cats (Bjegovic & Randic, 1971). The interference in central cholinergic activity by lithium may influence the consumption of ethanol. Other possible actions include the displacement of sodium ions from parts of the brain such as the hypothalamus by lithium ions (Ho & others, 1970) which may influence the osmoreceptors leading to a shift in water balance. Lithium is also known to influence the metabolism of monoamines, while ethanol preference in animals has also been attributed to tryptaminergic activities in the brain (Schildkraut, 1973; Myers & Veale, 1968). This work was supported by a grant in aid from the Licensed Beverage Industries, Inc. New York.

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REFERENCES

AMIT, Z., STERN, M. H. & WISE, R. S. (1970). *Psychopharmacologia*, **17**, 367–377.

BENTLEY, G. A. & SHAW, F. H. (1952). *J. Pharmac. exp. Ther.*, **106**, 193–199.

BJEGOVIC, M. & RANDIC, M. (1971). *Nature*, **230**, 587–588.

HEBB, C. (1963). In *Handbuch der exp. Pharmac.*, **15**, 55. Berlin: Springer.

HO, A. K. S., GERSHON, S. & PINCKNEY, L. (1970). *Archs int. Pharmacodyn. Ther.*, **186**, 54–65.

HO, A. K. S. & KISSIN, B. (1974). In *Proceedings of Second Int. Symp. on Alcoholism*, Manchester, U.K.

HO, A. K. S., SINGER, G. & GERSHON, S. (1971). *Psychopharmacologia*, **21**, 238–246.

MYERS, R. D. & VEALE, W. L. (1968). *Science, N.Y.*, **160**, 1469–1476.

SCHILDKRAUT, J. J. (1973). In *Lithium, its role in psychiatric research and treatment.* Editors: Gershon, S. and Shopsin, B. New York: Plenum Press.

SELLERS, E. M., COPPER, S. D. & ZILM, D. (1974). *The Proceedings of American Society for Clinical Pharmacology and Therapeutics* (March).

WREN, J. C., KLINE, N. S., COOPER, T. B., VARGA, E. & CANAL, O. (1974). *Clin. Med.*, **81**, 1, 33–36.

Modulation of the respiratory depressant effect of ethanol by 5-hydroxytryptamine

Most depressants of the central nervous system also depress respiration. We have measured respiratory depression induced in the mouse by alcohols, two barbiturates and an opioid using blood gas analysis (Hayashida & Smith, 1971) to determine whether their mechanisms of respiratory action differ. A strategy of neurotransmitter depletion and repletion was used.

Swiss-Webster female mice, 20–25 g, were used. Ethanol was diluted in normal saline to a concentration of 20% (w/v) and injected intraperitoneally (i.p.) in doses from 2 to 5 g kg^{-1}. These doses produced significant respiratory depression as indicated by a log-dose related rise in capillary blood P_{CO_2} and a fall in blood pH seen maximally 30 min after ethanol injection. (±)-Methadone (kindly donated by Mallinckrodt Chemical Works), sodium pentobarbitone, sodium phenobarbitone given in at least 3 doses for each drug were also injected 30 min before blood sampling. Reserpine (Serpasil) was given (i.p.) 4 h before other drugs. *p*-Chlorophenylalanine methyl ester was administered (300 mg kg^{-1}, i.p.) 4 h before ethanol injection. Blood was drawn into a 100 mm heparinized capillary tube without exposure to air from an incision made in the ventral surface of the proximal third of the tail. The blood was then transferred to a Radiometer blood micro system (BMS-3) and the pH and P_{CO_2} values displayed on a digital meter. The P_{O_2} values ranged from 75–90 mm Hg and were not dose-related to the depressant drug.

As shown in Table 1, ethanol significantly elevated capillary blood P_{CO_2} and lowered blood pH. Administration of reserpine or *p*-chlorophenylalanine (p-CPA) blocked the rise in P_{CO_2} normally induced by ethanol in a dose of 3 g kg^{-1} although blood pH remained depressed. The acidosis is probably attributable to the metabolic effect of the ethanol. The normal rise in P_{CO_2} induced by injections of various dosages of (±)-methadone or sodium pentobarbitone was however not blocked by the depletors of 5-hydroxytryptamine (5-HT). These findings suggest that 5-HT may specifically modulate the respiratory depression induced by ethanol.

Data also shown in Table 1 further implicate 5-HT; the respiratory-depressive effect of ethanol was restored in mice treated with reserpine and then injected intracerebrally with 10 μg of 5-HT. 5-HT was injected into the right cerebral hemisphere 15 min after ethanol in a volume of 10 μl using a microsyringe fitted with a guard to prevent penetration of the 25 gauge needle to a depth greater than 3 mm. Intracerebral injection of the saline vehicle or 5-HT alone did not produce any changes.

Since p-CPA inhibits tryptophan hydroxylase and prevents formation of 5-hydroxy-