inhibitor of $(Na^+ + K^+)$-ATPase and active ion transport[14,15] inhibited to the same extent ATPase activity of both normal and mutant mice. The effect of heat inactivation by preincubation at 50 °C for 15–60 min does not differ in groups examined.

The rather small decrease of the $(Na^+ + K^+)$-ATPase activity in Quaking mouse is not sufficient enough to allow a detailed comparison of the properties of the enzymes. In spite of this, the properties of the $(Na^+ + K^+)$-ATPase seem to be unchanged, as shown by similar pH optimum, similar heat inactivation pattern, inhibition by PCMB and fluoride of both control and mutant mice enzyme, as well as by the results on Km presented in this work. Crossed incubations performed with normal mouse plus Jimpy (another myelin-deficient mutant) brain homogenate gave no evidence of activation or inhibition effects on ATPase activity (unpublished). Hence, decrease of the $(Na^+ + K^+)$-ATPase activity here observed is more probably related to the quantity of the enzyme present.

The most evident feature of mutants is tremor recognized at about the 12th day after birth, the exact nature of which has not been clarified. Previous studies which have been done on brain adrenergic transmitters content[16] and turnover[17] indicated an accelerated dopamine metabolism.

Impaired transport of sodium and potassium in epileptic brain tissue was previously demonstrated[8]. According to WOODBURY[18], the sodium pump enzyme is involved in the action of anticonvulsive drugs. We report a decrease of the $(Na^+ + K^+)$-ATPase activity in some areas of brain of Quaking mouse which presents seizures after slight stimulation. The inborn error of this myelin deficiency in mice concerns the synthesis of the myelin constituents. It seems difficult to include directly the deficiency of the sodium pump enzyme in the genetic determined inborn error. It seems rather likely that the reduction of $(Na^+ + K^+)$ activated, ouabain sensitive ATPase activity in some areas of the brain of mutant mice is a secondary phenomenon, like the changes in adrenergic system observed mainly in older mice[17]. The mechanism underlying the impairment of $(Na^+ + K^+)$-ATPase activity is under investigation[19].

[16] J. P. TILLEMENT, M. C. DEBARLE, P. SIMON and J. R. BOISSIER, Experientia *27*, 268 (1971).

[17] E. KEMPF, J. GREILSAMER, G. MACK and P. MANDEL, J. Neurochem. *20*, 1269 (1973).

[18] D. M. WOODBURY, in *Basic Mechanisms of the Epilepsies* (Eds. H. H. JASPER, A. A. WARD and A. POPE; Little Brown and Co., Boston 1969), p. 647.

[19] Acknowledgement. We thank Miss M. OSTERTAG for skilfull technical assistance.

Morphine Suppression of Ethanol Withdrawal in Mice

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Summary. The acute administration of morphine, alcohol or dopamine results in a pronounced suppression of the convulsions produced by alcohol in mice. The suppressive action of morphine on alcohol withdrawal in the mouse apparently is not a product of morphine intoxication, but rather to some other specific interaction between alcohol and morphine in the central nervous system. The conclusion suggest that dopamine may play a significant role as a modulator in convulsions produced during alcohol withdrawal.

In spite of previous research[3–6] there is increasing evidence for a relationship between opiate and alcohol addiction[7–9]. There are reports of cross-tolerance between morphine and alcohol[10], blockade of ethanol dependence[9] and narcosis[11] in mice and there are reports of increased tolerance for morphine in mice[12] and in some human alcoholics that have been habituated to alcohol[13]. There is evidence for a connection between voluntary consumption of morphine and alcohol[8]. It has been found that a high % of opiate addicts that have graduated from drug free therapeutic communities become alcoholics five years later[14] and many opiate addicts have had earlier problems with alcohol drinking and often take large amounts of alcohol when narcotics are not available[15,16]. A strain of rats bred for high opiate consumption was found to drink more alcohol than a strain bred to ingest little morphine[17]. C 57 BL mice are known to

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[3] A. GOLDSTEIN and B. S. JUDSON, Science *172*, 290 (1971).

[4] P. V. HALUSKA and P. C. HOFFMAN, Science *169*, 1104 (1970).

[5] M. H. SEEVERS, Science *170*, 113 (1972).

[6] I. P. STOLERMAN, R. FUMAR and H. STEINBERG, Psychopharmacologia *20*, 321 (1971).

[7] D. H. Ross, M. A. MEDINA and H. LEE CARDENAS, Science *186*, 63 (1974).

[8] J. D. SINCLAIR, J. ATKINS and S. WALKER, Nature, Lond. *246*, 425 (1973).

[9] K. BLUM, J. D. EUBANKS and J. E. WALLACE, Proc. 37th Annual Meeting, Committee of Problems on Drug Dependence, Washington, D. C. (1975), paper submitted to Science, in press.

[10] D. H. Ross, National Council of Alcoholism Symposium, New York Academy of Science (1975), in press.

[11] K. BLUM, J. E. WALLACE, J. D. EUBANKS and H. A. SCHWERTNER, Pharmacologist Fall (1975).

[12] I. VENHO, R. EEROLA, E. V. VENHO and O. VARTIAINEN, Annls Med. exp. Biol. Fenn. *33*, 249 (1955).

[13] M. H. SEEVERS and G. A. DENENN, *Physiological Pharmacology* (Eds. W. S. ROOT and F. G. HORMANN, Academic Press, New York 1963), p. 565.

[14] R. G. SMART, personal communication.

[15] H. ISBELL, H. F. FRASER, A. WIKLER, R. E. BELLERILLE and A. J. EISENNAN, Q. Jl. Stud. Alcohol *16*, 1 (1965).

[16] H. KALANT and P. E. DENS, *Experimental Approaches to the Study of Drug Dependence* (Ed . H. KALANT and R. D. HAWKINS, Univ. of Toronto Press, Toronto 1969), p. 109.

[17] J. NICHOLS and S. HSIAO, Science *157*, 561 (1967).

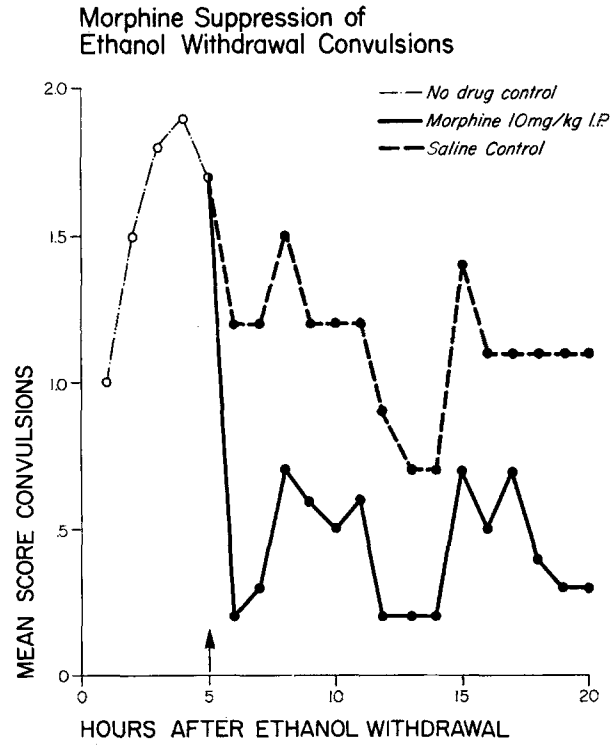

Fig. 1. Effects of morphine (10 mg/kg i.p.) induced withdrawal convulsions in mice. All *Ss* were tested for the first 5 h after the initiation of withdrawal in order to obtain non-drug treated control data.

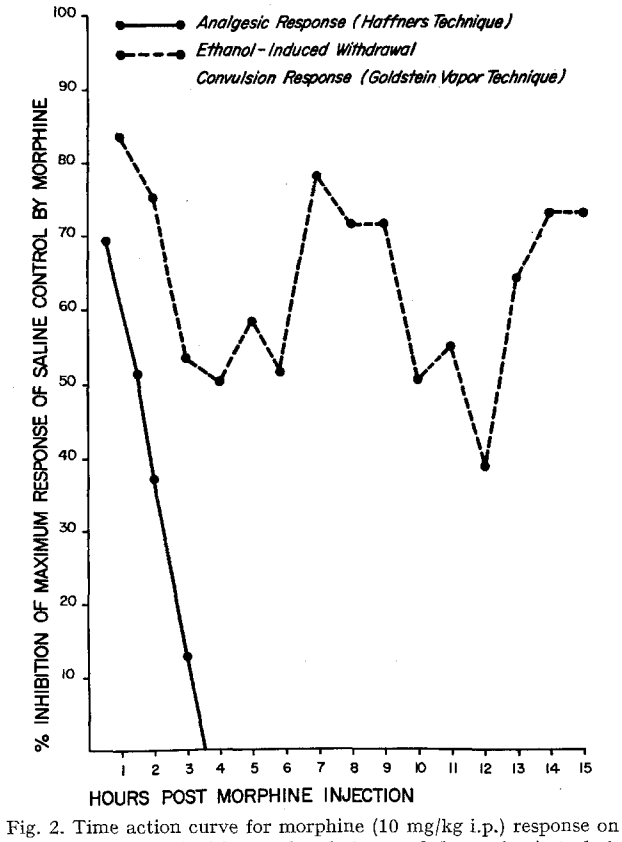

Fig. 2. Time action curve for morphine (10 mg/kg i.p.) response on the ethanol-induced withdrawal technique and the analgesic technique. The graph illustrates that the inhibitory effect of morphine on ethanol withdrawal are independent of morphine-induced CNS activity as measured by its analgesic response.

drink large amounts of both alcohol and morphine[18]. SINCLAIR[8] has shown a suppressive action of morphine on the drinking of alcohol.

The present studies were designed to evaluate whether morphine would influence alcohol-induced withdrawal convulsions in mice.

Swiss Webster mice were made physically dependent on ethanol by the GOLDSTEIN and PAL[19] inhalation technique. Mice were placed in an air-tight chamber and exposed to ethanol vapor for 3 days after which time they were abruptly withdrawn and measured for ethanol dependence. Dependence was defined in terms of resultant convulsion scores[3–6]. During the initial 3 days the mice were removed once a day for a 45 min period to permit the collection of blood samples and injection of 68 mg/kg of pyrazole to stabilize blood ethanol levels[20]. The mice were exposed to a vapor concentration of 21 mg/l for these 3 days and were then removed from the alcohol vapor chamber 24 h after their last dose of pyrazole. The grading system for assessing the severity of the withdrawal reaction has been described by GOLDSTEIN and PAL[19].

The method of HALEY and MCCORMICK[21] was used for intracerebral injections (i.c.) of drugs into conscious mice. The landmarks used for locating the site of injection were strictly adhered to and we noted the same behavioral effects: quietness for 1 min followed by normal activity after sham injections and artificial cerebral spinal fluid (CSF). A 27 guage needle was used in the injection procedure.

Separate groups of mice were given one of the following drug treatments: 1. Morphine sulfate 10 mg/kg (i.p.) after the 5th h post-ethanol withdrawal. Controls were given saline according to the same regimen. 2. Ethanol at 16 µg (i.c.) after the 5th h post-ethanol withdrawal. Controls were given cerebral spinal fluid (CSF) according to the same regimen. 3. Dopamine (i.c.) at 10 µg after the 1st h post-ethanol withdrawal. Blood alcohol was determined by a modification of the gas chromatographic procedure of WALLACE and DAHL[18].

Dopamine hydrochloride and ethanol were dissolved in artificial cerebral spinal fluid and injected intracerebrally (i.c.) at a volume of 10 µl[21]. The formula for the artificial CSF is as follows: NaCl 8.98 g/l, KCl 0.25 g/l, $CaCl_2$ 0.14 g/l, $MgCl_2$ 0.11 g/l, NaH_2PO_4 0.014 g/l, $NaHPO_4$ 0.066 g/l, urea 0.13 g/l and glucose 0.61 g/l. The pH of the solution was adjusted to 7.0 with 0.1 *N* NaOH. The dose of dopamine and morphine was calculated as the base rather than the salt form[22].

Figure 1 shows that morphine at a dose of 10 mg/kg (i.p.) significantly depressed ($p < 0.05$) the withdrawal convulsion scores of mice undergoing withdrawal from alcohol vapor exposure. Saline control scores were higher by 500% than those of the morphine treated mice at peak difference (Figure 1). The mean convulsion score was 1.11 whereas for morphine it was 0.43 showing marked suppression of ethanol withdrawal convulsions in mice. The duration of action of this effect for morphine was at least 15 h.

Is this suppression by morphine due to its short-term central depressant effects or to some other common specific interaction between alcohol and morphine in the

[18] K. ERIKSSON and K. KIIANMA, Annls Med. exp. Biol. Fenn. *49*, 73 (1972).
[19] D. B. GOLDSTEIN and N. PAL, Science *172*, 288 (1971).
[20] H. THEORELL, T. YORETANI and B. SJOBERG, Acta chem. scand. *23*, 255 (1969).
[21] B. K. KOE and S. S. TENEN, Biochem. Pharmac. *24*, 723 (1975).
[22] T. J. HALEY and W. G. MCCORMICK, B. J. Pharmac. *12*, 12 (1957).

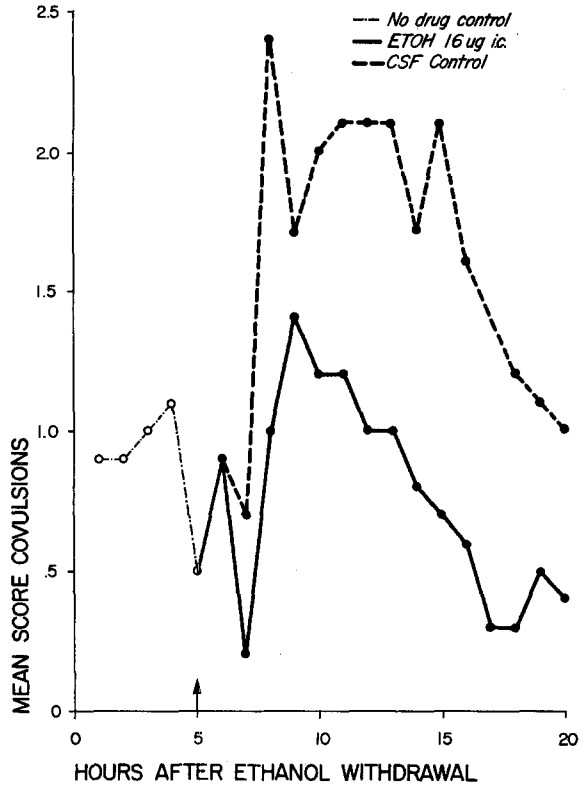

Fig. 3. Effects of ethanol (16 μg i.c.) on ethanol withdrawal response in mice. The drug was injected 5 h after the initiation of withdrawal.

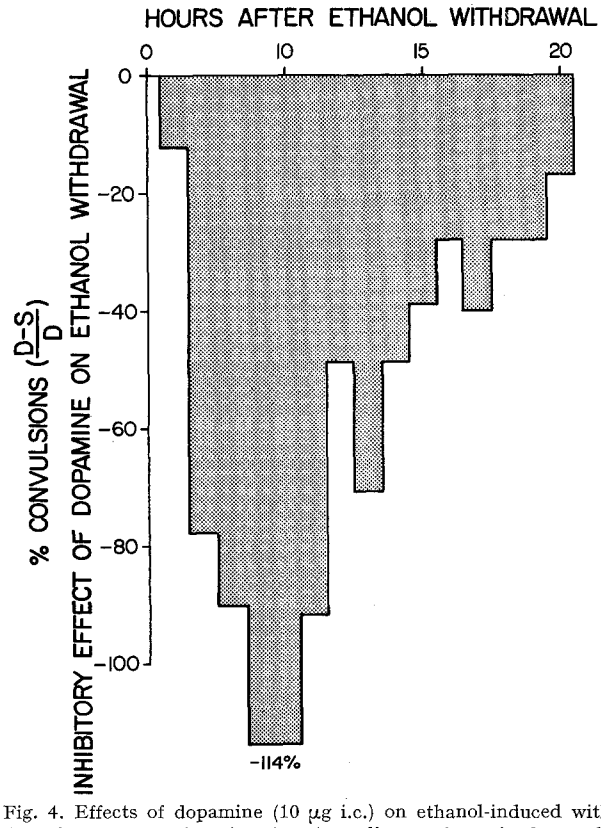

Fig. 4. Effects of dopamine (10 μg i.c.) on ethanol-induced withdrawal symptomatology in mice. According to the ratio the results are expressed as inhibitory effect of dopamine on ethanol withdrawal. The drug was injected at the 1st h post-alcohol withdrawal.

nervous system (CNS)? Experiments were designed to see if morphine at 10 mg/kg would produce analgesia as a measure of its central effects for a period shorter than 15 h.

The method of HAFFNER[23], as modified by BIANCHI and FRANCESCHINI[24] was employed to determine the duration of acute pharmacologic effects of morphine. In this procedure, an artery clip with the branches enclosed in a thin rubber tube, is applied to the root of the tail of a mouse for 30 sec; the animal makes continuous attempts to remove the noxious stimulus by biting the clip. In this experiment, there were 2 groups of 10 mice each. The mice were injected i.p. with either 10 mg/kg morphine sulfate or an equivalent volume of physiological saline and after 30 min the artery clip is applied for 30 sec.

Morphine at 10 mg/kg administered to naive mice produced a 69% inhibition of the biting response when compared to a saline control in the first 30 min after drug administration. After the 4th h, this analgesic response completely diminished. The analgesic response was significant only up to the 3rd h after the start of the experiment (Figure 2).

This experiment would suggest that the suppression observed on ethanol withdrawal convulsion response lasts much longer than morphine's short-term analgesic effects. Morphine suppressed ethanol-induced withdrawal convulsions by 83% at the 1st h following its injection and 15 h later the suppression is still at the 83% level compared to its saline control (Figure 2).

Administration of ethanol into the brain of mice undergoing withdrawal, significantly inhibited ($p < 0.005$) the convulsion response induced by abrupt removal of ethanol. With ethanol the scores were lower (367%) than those of its paired CSF control at peak difference. The mean convulsion scores for CSF was 1.60 ± 0.09 whereas for ethanol it was 0.77 ± 0.06 ($p < 0.005$) showing marked inhibition of ethanol dependence in mice (Figure 3).

The suppressive action of morphine on ethanol-induced withdrawal in mice does not appear to be due to morphine intoxication but due to some other common specific interaction between alcohol and morphine in the CNS. Other than the work by Ross et al.[7,10,25] showing a common depletion of regional brain calcium, the only common effect observed for morphine and ethanol involves brain dopamine. Acute administration of morphine was found to cause an accelerated depletion of brain dopamine after catecholamine synthesis inhibition, which was interpreted as an increased activity within the ascending dopamine from neurons[26]. SEEMAN and LEE[27] have shown that ethanol can induce a release of dopamine from neurons via a calcium-propagated coupling mechanism between the impulse and neurosecretion of dopamine. Other research in our laboratories show that haloperidol, a central dopamine receptor blocker[28], significantly intensifies ethanol-induced withdrawal convulsions in mice.

These findings suggests that morphine and ethanol-induced inhibition of ethanol withdrawal convulsions may be due to release or increased dopamine activity in the CNS following the administration of these agents.

[23] F. HAFFNER, Dt. Med. Wschr. *55*, 731 (1929).
[24] C. BIANCHI and J. FRANCESCHINI, J. Pharmac. *9*, 280 (1954).
[25] H. C. CARDENAS and D. H. ROSS, J. Neurochem. *24*, 487 (1975).
[26] L.-M. GUNNE, J. JONSSON and K. FUXE, Eur. Pharmac. *5*, 338 (1969).
[27] P. SEEMAN and T. LEE, J. Pharmac. exp. Ther. *190*, 131 (1974).
[28] N.-E. ARDEN, S. G. BUTCHER, H. CORRODI, H. FUXE and U. UNGERSTADT, Eur. Pharmac. *11*, 303 (1970).

Figure 4 illustrates the inhibitory ($p < 0.01$) effect of dopamine in terms of a ratio of

$$\frac{\text{D} - \text{L}}{\text{D}} \quad \frac{\text{dopamine convulsion} - \text{CSF convulsion}}{\text{dopamine convulsion}}.$$

According to this ratio, the intracerebral injection of dopamine resulted in a 157% inhibition of the withdrawal response at peak difference. The duration of action was at least 8 h, a finding similarly observed for morphine and alcohol.

Acute administration of morphine resulted in a marked inhibition of ethanol-induced withdrawal convulsions in mice. It is suggested on the basis of these in vivo experiments that dopamine may be an important link between morphine and ethanol actions. In vitro ethanol promotes the formation of tetrahydropapaveroline (THP), conjugate of dopamine with dihydroxyphenylacetaldehyde, which is the requisite intermediate in morphine biosynthesis in the opium poppy *Papaver somniferum*[28]. Evidence suggests that dopamine derived alkaloids are formed after the ingestion of ethanol[29] and may contribute to its addiction liability[30] and for neuropharmacological actions[31]. Other research shows[7,24] that morphine, ethanol and the dopamine conjugate of acetaldehyde (salsolinol) deplete regional brain calcium. This effect is selectively antagonized by the stereo-specific narcotic antagonist, Naloxone. Naloxone inhibits ethanol-induced withdrawal reactions[9] and also blocks the dopamine-derived alkaloid induction of seizure activity in mice[9]. Morphine abstinence results in diminished dopaminergic activity which is reversed to normal following Naloxone administration[25].

Morphine-induced suppression of ethanol withdrawal convulsions persists for a longer period of time than its duration of analgesia; thus suggesting that the inhibitory effect of morphine is not due to its intoxication but rather to some other specific interaction between ethanol and morphine[32].

It is possible that dopamine may play a significant role as a modulator in the withdrawal convulsions induced by ethanol and may even account for morphine suppression of this convulsion response. Other biochemically directed research may eventually delineate this interaction between morphine and ethanol and provide a further rationale for its treatment of withdrawal symptomatology.

[29] V. E. DAVIS and M. J. WALSH, Science *167*, 1005 (1970).
[30] M. A. COLLINS and M. G. BIGDEL, Life Sci. *16*, 585 (1975).
[31] G. COHEN, *Frontiers in Catecholamine Research* (Pergamon Press, Oxford 1974), vol. 173, p. 1021.
[32] K. BLUM, J. D. EUBANKS, J. E. WALLACE, W. W. MORGAN and H. A. SCHWERTNER, N.Y. Acad. Sci. NCA Symposium on Alcoholism (1975), in press.

The Influence of Lithium on Serum Ceruloplasmin

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Summary. Serum ceruloplasmin oxidase activity did not change in mice treated orally for 120 days with Li_2CO_3 (0.58 mEq/kg/day). After a single i.p. injection of LiCl (20 mEq/kg), a significant activation of ceruloplasmin was observed.

It is well established that lithium carbonate is a prominent therapeutic agent against manic-depressive psychosis[2,3], but the mechanism of lithium effects is still an open question[4,5]. According to our bibliographic search reports on the effect of Li^+ on ceruloplasmin are lacking. Since this plasma oxidase may be involved in some forms of mental disease[6] and in the mechanism of action of centrally active drugs[7], the present study was conducted in an attempt to examine the possible influence of Li^+ on ceruloplasmin activity.

Material and methods. Male Swiss mice (mean body weight 29 g) maintained on a standard balanced diet ad libitum were used. In long term experiments mice received as drinking water a solution containing 100 mg of Li_2CO_3/l. To the control group distilled water was given. After 120 days of treatment the mice were killed by decapitation, blood samples were collected and the sera separated and stored at −20 °C. The maximum storage time was 24 h. Ceruloplasmin oxidase activity was determined by the method of RAVIN[8] and values expressed in mg/100 ml of serum. In experiments with LiCl or NaCl, a *M* solution was administered i.p. at the dose of 20 mEq of Li^+ or Na^+/kg and blood samples were taken by decapitation 2 h after the injections. The sera were separated, stored and ceruloplasmin determined as above. The significance of the differences were evaluated by the Student *t*-test[9].

Results and discussion. The mean daily intakes of water or Li_2CO_3 solution throughout the period of experimentation were 6.3 and 6.4 ml/mouse, respectively. This dose corresponds to 0.58 mEq lithium/kg body weight/day.

From the results presented in the Table, it is clear that long-term administration of Li_2CO_3 does not change the levels of ceruloplasmin, since the difference between Li^+ treated and control mice is not significant ($t=1.565$, $p < 0.2$). The dose of 20 mEq/kg NaCl does not have any influence on ceruloplasmin, since the difference between these mice and the normal controls (Table) is not significant ($t=0.559$, $p > 0.5$). In contrast to these findings, the group of mice treated i.p. with LiCl presented a ceruloplasmin activity higher than the NaCl-injected

[1] R. R. A. is working with a research grant from the Conselho Nacional de Pesquisas (National Research Council of Brasil).
[2] M. SCHOU, Pharmac. Rev. *9*, 17 (1957).
[3] P. C. BAASTRUP and M. SCHOU, Archs. gen. Psychiat. *16*, 162 (1967).
[4] S. GERSHON, A. Rev. Med. *23*, 439 (1972).
[5] D. SAMUEL and Z. GOTTESFELD, Endeavour *32*, 122 (1973).
[6] S. PUZYŃSKI and A. KALINOWSKI, Nature, Lond. *212*, 399 (1966).
[7] B. C. BARRASS and D. B. COULT, Biochem. Pharmac. *21*, 677 (1972).
[8] H. A. RAVIN, J. Lab. clin. Med. *58*, 161 (1961).
[9] G. W. SNEDECOR, *Statistical Methods* (Iowa State College Press, Ames, Iowa 1962).