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Cross-Tolerance to Phenobarbital Following Chronic Ethanol Polydipsia[1]

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LAU, C. E., M. TANG AND J. L. FALK. *Cross-tolerance to phenobarbital following chronic ethanol polydipsia.* PHARMAC. BIOCHEM. BEHAV. 15(3) 471–475, 1981.—Using contingent food pellet delivery, rats were trained on a discriminative motor control task requiring that a force transducer be held steadily within a force band. Motor performance following pre-task doses of phenobarbital (40, 60 or 80 mg/kg) both before and after 4 months of chronic ethanol polydipsia (mean intake=11.1 g/kg/day) indicated the development of cross-tolerance from ethanol to phenobarbital. Days on which saline control injections were given in place of phenobarbital injections (on injection days ethanol was withdrawn 5 hr pre-injection) revealed the development of a mild physical dependence on ethanol at this level of ethanol polydipsia. Chronic ethanol polydipsia did not alter the time course of phenobarbital elimination from the serum, indicating that the cross-tolerance probably was due to central nervous system changes.

Barbiturate cross-tolerance Ethanol polydipsia Phenobarbital Schedule-induced polydipsia Ethanol physical dependence

COMMON clinical lore claims that alcoholics, when sober, are unusually resistant to hypnotic and sedative agents. However, systematic studies of ethanol-barbiturate cross-tolerance are lacking and the effects of agitation due to ethanol withdrawal need to be ruled out. Aside from questions concerning the proper dosage of alcoholic patients to achieve sedation or anesthesia, the study of cross-tolerance could illuminate the hazards of combined use of alcohol and barbiturates. Epidemiologic and clinical laboratory investigations indicate both considerable barbiturate abuse in alcoholics and unexpected blood ethanol levels in drug intoxicated persons [3,9].

In the present study, we chose to use the slowly-metabolized barbiturate phenobarbital so that any change in tolerance to this drug after chronic exposure to ethanol would probably not be attributable to a change in drug degradation, especially since behavioral testing was to occur not long after phenobarbital administration. The technique of schedule-induced ethanol polydipsia [5,6] was used to produce chronic ethanol overdrinking in rats in order to estimate cross-tolerance to phenobarbital. The behavioral response to phenobarbital was evaluated using a discriminative motor control procedure [4, 16, 24], both prior to and following chronic exposure to ethanol.

METHOD

Animals

Eight male, albino, Holtzman rats (mean body weight =365 g, range: 343–396 g) were used in the present study. They were housed initially in individual, stainless steel Acme cages in a temperature-controlled room with a 12-hr on, 12-hr off light cycle. Tap water was available at all times.

Drugs

Sodium phenobarbital (Ayerst Lab., Inc., New York, NY) injection solution was prepared by dissolving 40 mg of the sodium salt in 1 ml of isotonic saline. Solutions were always made immediately before the injection. A 5% (v/v) ethanol solution was made available for drinking during one phase of the experiment.

Apparatus

The motor task evaluation apparatus consisted of a Plexiglas chamber (25×30×30 cm) with stainless steel front and rear panels. A stainless steel manipulandum rested on a force transducer unit (Statham Model UC3 strain gauge mounted on a Statham Model UL4 load cell) and was coupled to a bridge amplifier (Statham Model SC1105) that connected directly into a Lab-8 digital computer (Digital Equipment Corp.). A food pellet receptacle was mounted on the same panel as the manipulandum but with enough distance (17 cm) between them to prevent an animal from touching the manipulandum and reaching into the food tray simultaneously. The manipulandum was shielded so an animal could only touch it with a single paw. At the top of this panel an audio generator (Sonalert, Mallory) was mounted which was used to provide an audio feedback tone when the force applied to the lever was within the required limits (see below). Details of the apparatus have been described previously [16,24].

Procedure

Discriminative motor control training. Animals were food deprived (fed only 5 g/day) for 3 days. Daily training on the discriminative motor task was started on the fourth day. Four animals were trained initially to hold the manipulandum

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for 0.5 sec within a wide force-band limit (10–30 g). The computer was programmed to deliver a 45-mg Noyes food pellet into the pellet receptacle after each 0.5 sec lever hold within the above force limits. The final parameters for the task required a continuous application of force within a 15–30 G force band for 1.5 sec. Each animal's session was terminated after the delivery of 50 pellets or if a 1/2 hr pause in performance occurred. All animals were given sessions every other day until motor performance stabilized which took an average of 4 months. Motor performance was evaluated by four measures that were calculated from the session values.

(A) In-band efficiency: $\frac{\text{minimum possible in-band time}}{\text{in-band time}}$

(B) Tonic accuracy: $\frac{\text{in-band time}}{\text{total response time}}$

(C) Work rate: $\frac{\text{total response time}}{\text{session time}}$

(D) Dyskinesia: total number of entrances into force band.

Food supplements (Purina Lab Chow, pelleted) necessary to maintain the animals at 80% of their starting body weights were given in the home cage. On days when the animals received training sessions, the ration was given immediately after the session.

Initial phenobarbital dose-response determination. After steady baseline performance was attained, animals were given subcutaneous injections of 0, 20, 40, 60 and 80 mg/kg of sodium phenobarbital 1.5 hr pre-session in a random order. Each dose was given at least twice, and a minimum of 7 days was allowed between injections.

Chronic ethanol administration. After the initial phenobarbital dose-effect determinations, the animals were transferred into individual, Plexiglas chambers (27×30×24 cm) in a continuously-illuminated room. Each chamber was equipped with a stainless-steel food receptacle. Drinking fluid was available continuously from a stainless-steel, ball-bearing drinking spout (Ancare TD-300) which was attached to a 250-ml Nalgene graduated cylinder. A 45-mg food pellet was delivered automatically every 2 min for 1 hr, giving a total of 30 pellets per feeding session. This was followed by a 3-hr period with no food. Thus, there were six 1-hr feeding sessions every 24 hr. A 5% ethanol solution was available as the sole drinking fluid.

Phenobarbital dose-response redetermination under chronic ethanol administration. After the animals had been ingesting alcohol chronically for 4 months, their phenobarbital dose-response relation on discriminative motor control was redetermined. To insure a zero blood alcohol level at the time of barbiturate administration, isocaloric glucose or water was substituted for ethanol as the drinking fluid 5 hr before an injection. As in the initial dose-response determination, all phenobarbital doses were given 1.5 hr pre-session. Thus, performance under a 0 mg/kg dose is actually that of a 6.5 hr ethanol withdrawal.

Blood ethanol and phenobarbital level determinations. Small (100 μl) tail blood samples were used for determining the blood levels of both drugs. Blood ethanol levels were determined twice within a 24-hr period (0700 and 1900 hr) in all animals 3 weeks before the phenobarbital dose-response redetermination was initiated. Ethanol concentrations were measured with a Perkin-Elmer 3920 B gas chromatograph according to the method developed by LeBlanc [13]. Serial blood samples for serum phenobarbital determinations were obtained from 4 rats at 1, 1.5, 2 and 3 hr post-injection and analyzed by a gas chromatographic technique previously described [12]. These 4 animals were exposed to conditions identical to those given the animals trained on the discriminative motor control task, except they were not given this training. Serum phenobarbital levels were measured after doses of 40, 60 and 80 mg/kg and redetermined at the 60 and 80 mg/kg doses after the animals were exposed to the chronic alcohol treatment for 4 months.

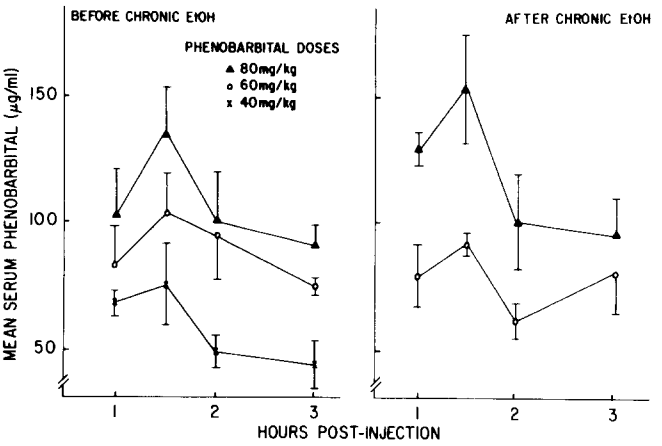

FIG. 1. Time course of mean (±S.E.) serum phenobarbital level (μg/ml) in rats (N=4) as a function of dose (SC) before and after 4 months of chronic schedule-induced ethanol (EtOH) drinking.

RESULTS

Figure 1 shows the mean serum concentrations of phenobarbital in food-limited rats before (left) and after (right) exposure to 4 mon of chronic ethanol ingestion. Data in the left panel have been reported previously [25]. Serum phenobarbital concentrations were positively correlated with the size of the dose administered during the entire 3 hr. For all 3 doses studied (40, 60 and 80 mg/kg) serum drug levels peaked 1.5 hr post-injection. Four mon of chronic ethanol drinking did not alter the time when the highest concentration of barbiturate was found in the serum, although substantially higher 1 and 1.5 hr values were obtained after the 80 mg/kg dose.

Table 1 gives the results of the chronic ethanol drinking procedure for the animals trained on the discriminative motor control task. The two times at which blood was sampled for ethanol determination were at a morning and evening point 2 hr after a feeding period (i.e., 1 hr before the next feeding period). Since ethanol drinking is associated mainly with the food-schedule periods, the above blood sampling times give a conservative estimate of the blood ethanol level maintained by this procedure [6].

The influence of various doses of phenobarbital on the indices of discriminative motor control before the animals had received chronic exposure to ethanol is shown in Fig. 2. In-band efficiency decreased by almost 50% throughout most of the dosage range. Tonic accuracy began decreasing at 40 mg/kg and decreased further with increasing dose levels. Work rate was unaffected until the 60 mg/kg level. The actual magnitude of the decrease in work rate is somewhat arbitrary because if an animal paused for one-half hr this was set as the criterion for terminating a session. This happened on 3 occasions at the 60 and on 2 occasions at the 80 mg/kg dose.

TABLE 1

SCHEDULE-INDUCED ETHANOL INTAKES AND RESULTING BLOOD ETHANOL CONCENTRATIONS

Animal	Daily EtOH Intake (g/kg)*	Blood EtOH Contentration (mg/dl)	
		0700 hr	1900 hr
J 2	12.3	103.6	66.0
J14	10.1	41.0	70.4
J17	12.7	160.0	154.8
K19	9.3	118.2	125.0
Mean	11.1	105.7	104.9

*Each value is a mean of the 10 days before blood samples were taken.

Dyskinesia increased as a function of dose level up to 60 mg/kg. At 80 mg/kg, one animal (J2) failed to work and one (J14) actually decreased in dyskinesia. The phenobarbital dose-effect relations obtained after the chronic ethanol drinking phase of the experiment are not presented. Changes in the incidence of 1/2-hr pauses at the higher doses obviated intra-animal comparisons since the calculated values would represent very different numbers of session pellets earned. Consequently, an analysis in terms of changes in the number of pellets earned at each dose level before and after chronic ethanol ingestion is presented.

Figure 3 shows for each animal the mean number of pellets earned in a session (maximum is 50) as a function of pre-session phenobarbital dose both before and after chronic ethanol ingestion. For two animals, J2 and J17, the sharp decrease in pellets earned in a session which occurred at the 60 mg/kg dose was shifted to 80 mg/kg after the 4 months of chronic ethanol exposure, indicating the development of cross-tolerance from ethanol to phenobarbital. Animal K19 did not decrease the number of pellets earned at even the 80 mg/kg dose either before or after chronic exposure to ethanol. Animal J14 showed the sharp decrease in pellets earned at the 80 mg/kg dose only after chronic ethanol exposure. This apparent reversal in the direction of the results is explicable in terms of serum phenobarbital level. After the discriminative motor control session on representative drug-injection days, blood samples were drawn at 1.35–2 hr post-injection for serum phenobarbital determination. These results confirmed the data shown in Fig. 1 and are not presented, but the trend for animals given the largest dose (80 mg/kg) to exhibit greater peaks in serum phenobarbital after chronic ethanol exposure was notable in J14. Two hr post-injection serum phenobarbital level was 156.6 μl/ml, an elevated value which might well explain the decrease in pellets earned at that dose. Consequently, unless the serum phenobarbital became excessively elevated as a result of chronic, previous exposure to ethanol, then changes in pellets earned per session revealed a cross-tolerance effect between ethanol and phenobarbital.

In the cross-tolerance testing procedure, animals were withdrawn from ethanol for 5 hr before being administered a drug dose to ensure a zero blood alcohol level and hence no synergism of phenobarbital by ethanol. They were given the motor task, as usual, 1.5 hr post-injection. When the injection was a saline control, rather than a dose of phenobarbital,

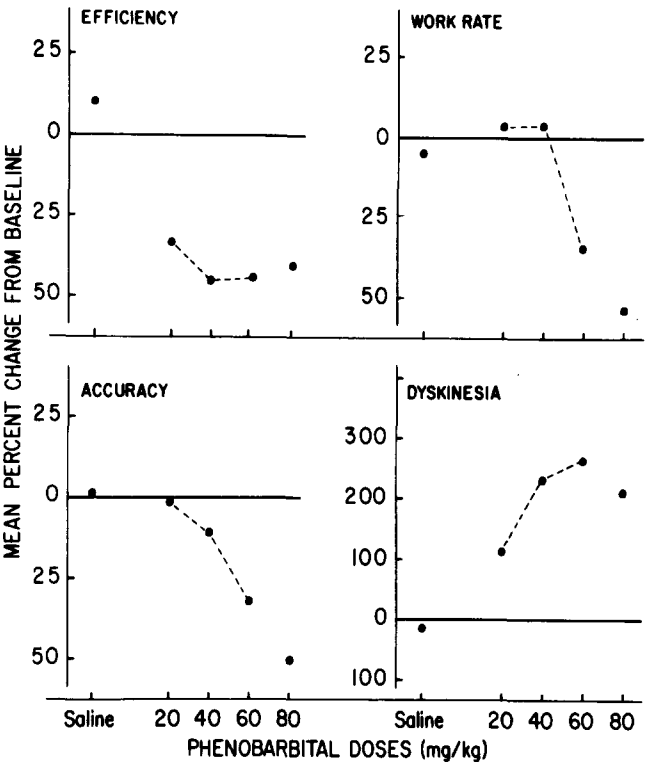

FIG. 2. Mean percent change from baseline performance on four indices of discriminative motor control in rats (N=4) as a function of phenobarbital dose (SC). Baseline is mean of 3–4 sessions immediately preceding a particular dose. The unjoined points at 80 mg/kg are based on the performance of only 3 rats since the fourth animal had shown large pauses at the next lower dose (60 mg/kg) and was not given the highest dose.

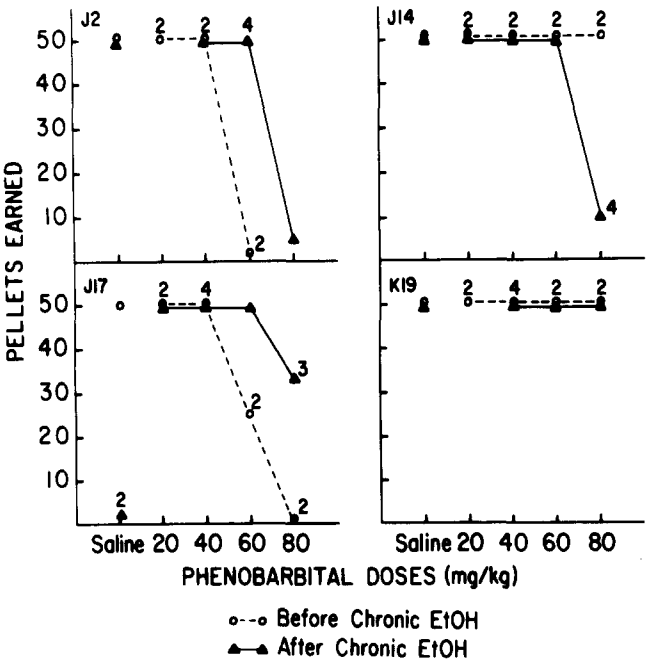

FIG. 3. Number of pellets earned in a session for each rat (N=4) as a function of phenobarbital dose (SC) before and after 4 months of chronic schedule-induced EtOH drinking. Numbers adjacent to plotted points indicate number of replications.

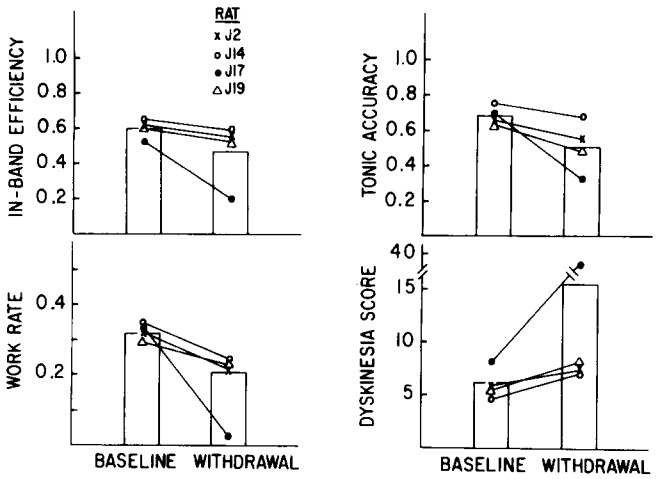

FIG. 4. Mean motor performance (plotted points indicate individual animal data) on four indices of discriminative motor control. Baseline is mean of 3–4 sessions immediately preceding a withdrawal session. Withdrawal constituted 6.5 hr without access to EtOH solution.

this procedure amounted to a 6.5 hr withdrawal from chronic ethanol drinking. Such a withdrawal might be expected to produce a decrement in discriminative motor control and this is borne out by Fig. 4. While one animal (J17) clearly was affected to a greater degree than the others, all animals showed the same trends: in-band efficiency, tonic accuracy and work rate all decreased and dyskinesia increased. The randomization test for matched pairs ([21], pp. 89–91) reveals that each of these motor measures under withdrawal is different from its respective baseline condition at the $p=0.06$ level (one-sided test). While this significance value is slightly greater than the commonly accepted criterion, it is nevertheless of interest that the 0.06 level is attained with an N of only 4 animals and that there were no reversals in the trend of these data.

DISCUSSION

The time course of phenobarbital disappearance from the blood (Fig. 1) was not greatly altered by a history of chronic ethanol ingestion. Phenobarbital is long-acting and only slowly metabolized [29], and chronic ethanol exposure did not change this picture. Other studies on the rat also found no change in the disappearance of the short-acting, more rapidly metabolized pentobarbital after prior chronic ethanol ingestion [7,10].

The dose-effect relations on discriminative motor control produced by phenobarbital (Fig. 2) were similar to those obtained on a previous study using pentobarbital [4]. In-band efficiency, dyskinesia and tonic accuracy all deteriorate as a function of dose level. Work rate decreases only at the higher dose levels. The rotarod test of motor coordination also showed impairment only at high dose levels of pentobarbital and amobarbital in mice [27]. Using phenobarbital, Kelleher *et al.* [11] found a decrease in DRL 18 sec response rates in rats only at the highest dose given (80 mg/kg), but not even this dose decreased fixed ratio 50 rates.

Straight-alley running times in rats were not much impaired by a 60 mg/kg injection of phenobarbital [19]. Similarly, in previous research we found that fixed-interval one-min rates in rats were decreased only at 80 mg/kg phenobarbital [23]. However, in that study doses of phenobarbital from 20 to 80 mg/kg decreased the schedule-induced polydipsia generated by the fixed-interval schedule in a graded fashion.

In behavioral situations requiring sustained individual responses, sustained key-holding in dogs [28] and postural stasis in pigeons [2], comparatively low doses of pentobarbital affected performance by shortening response duration. Likewise, the discriminative motor control situation required a sustained response and was sensitive to low doses of barbiturates.

The mean daily ethanol intake during the chronic, schedule-induced drinking phase (11.1 g/kg/day, cf. Table 1) was somewhat lower than the intakes we typically attain with this technique. Although we usually attain about 13 g/kg/day [6, 17, 18, 22], we [16,24] as well as other investigators [8] have sometimes attained values between about 10 and 12 g/kg/day. Nevertheless, even within this lower intake range, motor dyskinesia [16,24] and running fits [8] were evident upon withdrawal in those studies, indicating the induction of physical dependence. When a saline control injection was given in the cross-tolerance testing phase of the present experiment, the procedure was, in effect, a 6.5 hr withdrawal from chronic ethanol polydipsia. As shown in Fig. 4, all mean measures of motor competence declined moderately, indicating a borderline physical dependence on ethanol.

Studies on possible cross-tolerance from ethanol to barbiturates have used mainly agents which are subject to significant metabolic degradation (e.g., amobarbital, hexobarbital and pentobarbital). Human alcoholic patients had elevated sedation thresholds and recovery times to amobarbital administration [14]. Another study did not find evidence of a higher sedation threshold to amobarbital in alcoholic patients, but these subjects may have recovered from their alcohol dependence by the time of testing [20]. The behavioral response studied in animal research has been limited to a change in barbiturate sleeping time resulting from a history of chronic exposure to ethanol. A history of chronic ethanol adminsitration in rats produced a decreased sleeping time to pentobarbital [1,10] and to amobarbital [15]. This evidence of cross-tolerance from ethanol to the short-acting barbiturates was confirmed in another sleeping-time study using the long-acting agent phenobarbital [7]. Using an EEG burst suppression measure, Wahlstrom [26] found cross-tolerance from ethanol to hexobarbital. The present results strongly suggest that cross-tolerance occurs between ethanol and the long-acting barbiturate phenobarbital on a behavioral measure other than sleeping time.

REFERENCES

1. Ahlquist, R. P. and J. M. Dille. Reactions of alcohol tolerant rabbits to pentobarbital, evipal, ether, amidopyrine and metrazol. *J. Pharmac. exp. Ther.* **70**: 301–308, 1940.
2. Blough, D. S. New test for tranquilizers. *Science* **127**: 586–587, 1958.
3. Devenyi, P. and M. Wilson. Abuse of barbiturates in an alcoholic population. *Can. med. Ass. J* **104**: 219–221, 1971.
4. Falk, J. L. Drug effects on discriminative motor control. *Physiol. Behav.* **4**: 421–427, 1969.

5. Falk, J. L. and H. H. Samson. Schedule-induced physical dependence on ethanol. *Pharmac. Rev.* **27:** 449–464, 1975.
6. Falk, J. L., H. H. Samson and G. Winger. Behavioral maintenance of high concentrations of blood ethanol and physical dependence in the rat. *Science* **177:** 811–813, 1972.
7. Hatfield, G. K., T. S. Miya and W. F. Bousquet. Ethanol tolerance and ethanol-drug interactions in the rat. *Toxic. appl. Pharmac.* **23:** 459–469, 1972.
8. Heintzelman, M. E., J. Best and R. J. Senter. Polydipsia-induced alcohol dependency in rats: A reexamination. *Science* **191:** 482–483, 1976.
9. Hirsch, C. S., J. C. Valentour, L. Adelson and I. Sunshine. Unexpected ethanol in drug-intoxicated persons. *Postgrad. Med.* **54:** 53–57, 1973.
10. Kalant, H., J. M. Khanna and J. Marshman. Effect of chronic intake of ethanol on pentobarbital metabolism. *J. Pharmac. exp. Ther.* **175:** 318–324, 1970.
11. Kelleher, R. T., W. Fry, J. Deegan and L. Cook. Effects of meprobamate on operant behavior in rats. *J. Pharmac. exp. Ther.* **133:** 271–280, 1961.
12. Lau, C. E., M. Tang and J. L. Falk. Micro-sample gas-chromatographic technique for the analysis of barbiturates. *Pharmac. Biochem. Behav.* **11:** 355–357, 1979.
13. Le Blanc, A. E. Microdetermination of alcohol in blood by gas-liquid chromatography. *Can. J. Physiol. Pharmac.* **46:** 665–667, 1968.
14. Mellor, C. S. Sedation threshold and sedation recovery time in alcoholics. *Biol. Psychiat.* **9:** 125–130, 1974.
15. Ratcliffe, F. The effect of chronic ethanol administration on the responses to amylobarbitone sodium in the rat. *Life Sci.* **8:** 1051–1061, 1969.
16. Samson, H. H. and J. L. Falk. Ethanol and discriminative motor control: Effects on normal and dependent animals. *Pharmac. Biochem. Behav.* **2:** 791–801, 1974.
17. Samson, H. H. and J. L. Falk. Schedule-induced ethanol polydipsia: Enhancement by saccharin. *Pharmac. Biochem. Behav.* **2:** 835–838, 1974.
18. Samson, H. H. and J. L. Falk. Alteration of fluid preference in ethanol-dependent animals. *J. Pharmac. exp. Ther.* **190:** 365–376, 1974.
19. Schmidt, H., Jr. and A. L. Stewart. Acute effect of phenobarbital upon a locomotor response. *Physiol. Behav.* **2:** 403–407, 1967.
20. Shagass, C. and A. L. Jones. A neurophysiological study of psychiatric patients with alcoholism. *Q. Jl Stud. Alcohol* **18:** 171–182, 1957.
21. Siegel, S. *Nonparametric Statistics for the Behavioral Sciences.* New York: McGraw-Hill, 1956.
22. Tang, M. and J. L. Falk. Ethanol dependence as a determinant of fluid preference. *Pharmac. Biochem. Behav.* **7:** 471–474, 1977.
23. Tang, M. and J. L. Falk. Behavioral and pharmacological components of phenobarbital tolerance. In: *Behavioral Tolerance: Research and Treatment Implications, NIDA Research Monograph No. 18,* edited by N. A. Krasnegor: Washington DC: U.S. Govt. Printing Office, 1978, pp. 142–148.
24. Tang, M. and J. L. Falk. Ethanol withdrawal and discriminative motor control: Effect of chronic intake level. *Pharmac. Biochem. Behav.* **11:** 581–584, 1979.
25. Tang, M., C. E. Lau and J. L. Falk. Serum phenobarbital and barbital concentrations in rats on a limited food regimen. *Pharmac. Biochem. Behav.* **11:** 359–361, 1979.
26. Wahlstrom, G. Changes in a hexobarbital anesthesia threshold in rats induced by repeated long-term treatment with barbital or ethanol. *Psychopharmacologia* **19:** 366–380, 1971.
27. Watzman, N. and H. Barry, III. Drug effects on motor coordination. *Psychopharmacologia* **12:** 414–423, 1968.
28. Weiss, B. and V. G. Laties. Effects of amphetamine, chlorpromazine, pentobarbital, and ethanol on operant response duration. *J. Pharmac. exp. Ther.* **144:** 17–23, 1964.
29. Williams, R. T. and D. V. Parke. The metabolic fate of drugs. *A. Rev. Pharmac.* **4:** 85–114, 1964.