Pharmacology Biochemistry & Behavior, Vol. 10, pp. 329–334. Printed in the U.S.A.

Relations Between Metabolic and Nervous Tolerance Toward Ethanol in Naive and Chronically Intoxicated Rats[1]

DOM MICELI AND JACQUES LE MAGNEN

Laboratoire de Neurophysiologie Sensorielle et Comportementale
Collège de France, 11, place Marcelin Berthelot 75231 Paris Cedex 05 France

(Received 30 October 1978)

MICELI, D. AND J. LE MAGNEN. *Relations between metabolic and nervous tolerance towards ethanol in naive and chronically intoxicated rats.* PHARMAC. BIOCHEM. BEHAV. **10**(3) 329–334, 1979.—Interindividual differences in blood alcohol elimination rates indicating metabolic tolerance (MT) were determined in adult male Wistar rats following the injection of ethanol via chronically implanted intra-jugular catheters. Nervous tolerance (NT) was measured using a behavioral test based on the latency of drinking in 20-hr water-deprived rats following the IV injection of a challenge dose of ethanol. Individual elimination rates varied between 0.312 and 0.570 mg/ml/hr (mean ± SE: 0.427 ± 0.006) and drinking latencies (1/NT) between 45 and 255 min (mean=125 ± 12). MT and NT determinations performed on the same animals indicated no correlation between the initial degrees of tolerance. In one experiment, MT and NT measurements were obtained prior to and following chronic IV pulsed-administration (4 days) of either ethanol or physiological saline. In comparison to controls, the ethanol-treated rats showed both a significant increase (22.7%) in MT ($p<0.01$) and a reduction (66.8%) in the latency (1/NT) of drinking ($p<0.05$). The extrapolation of BALs present at the onset of drinking indicated the concomitant increase in MT of ethanol-treated animals to be negligible in accounting for the observed increase in NT and that the latter was more likely attributable to adaptive changes in CNS sensitivity. Another experiment attempted to investigate the time course of development of MT in rats during continuous IV ethanol infusion (5–9 days). Mean daily MT values were calculated on the basis of 24-hr BAL samplings and known quantities of ethanol administered. The results indicated an initial adaptive increase in MT occurring within 48 hr and attaining rates about as high as 35% above initial values. This phase was usually followed by a rapid decrease in MT below that of initial levels and associated with elevated BALs and loss of body weight due to reduced food intake. The possible role played by the MT and NT factors in the defense mechanisms involved in preference and aversion conditioning and regulating the voluntary consumption of ethanol in the naive and chronically intoxicated animal are discussed.

Ethanol metabolism Ethanol nervous tolerance Chronic ethanol treatment Intravenous ethanol infusion

ALCOHOL dependence does not develop in the majority of human subjects and in most animal species offered ethyl alcohol since the amounts ingested are less than those which would ultimately produce symptoms of chronic intoxication. One possible explanation for such self-controlled intake is linked to the physiological mechanisms regulating the selection of food and fluids and involve the conditioning of preferences and aversions depending on the post-ingestive effects [37]. In the latter control process regarding ethanol, associated with its aversive central effects [2, 3, 17], two critical and genetically determined factors appear to be the metabolic and nervous tolerance which the organism manifests toward ethanol. Whereas metabolic tolerance (MT) defines the capacity for ethanol elimination and thereby the likelihood of the drug to exert its effects on the target receptors at the CNS level, nervous tolerance (NT) reflects basic CNS sensitivity to the ethanol stimulus. It is therefore important to examine the manner in which these tolerance factors [12] influence the establishment of the pathophysiological process, the subsequent modifications which they undergo during chronic intoxication and their interrelations at the various stages in the induction of physical dependence.

Several studies have indicated identical MTs to be found among animals or strains exhibiting rather different degrees of NT [8, 34, 35] whereas other data have demonstrated a positive correlation between MT and NT in the naive animal [1]. Similarly inbred strains of mice differing on the basis of ethanol-induced sleep times [10] have been shown to exhibit an inverse relationship between this parameter and the level of hepatic alcohol dehydrogenase activity [36,43]. In man there is evidence of marked intra- and inter-racial differences in sensitivity towards alcohol, such as the greater alcohol intolerance displayed by Orientals compared to Occidentals; however, the physiological basis for these differences has yet to be clearly elucidated [4, 6, 32, 39].

Besides the study of initial relations between MT and NT in the naive subject, other investigations have focused on the respective alterations in the two tolerances in response to chronic ethanol administration. Although it is commonly accepted that NT increases after prolonged ethanol treatment, data regarding MT have been more divergent, with reports of

[1]This work was supported by an IREB research grant.

increases ([7, 18, 24, 46] rat; [13, 23, 25] man) and no change ([21,38] rat; [22] man).

The aim of the present investigations was to re-examine in the same rats both the initial relations between NT and MT and the changes in tolerance resulting from the chronic intravenous (IV) administration of ethanol. The intravenous route offered precise control of the parameters of intoxication (dose, frequency) thereby standardizing the treatments. Moreover, by using a continuous IV infusion paradigm, the daily MT levels could be calculated on the basis of single daily BAL samplings and known quantities of ethanol administered. Consquently the time course of development of MT could be studied throughout the chronic intoxication period.

METHOD

The experiments were performed on adult male Wistar rats weighing between 300 and 479 g (aged 12–22 weeks) depending on the experiment. They were individually housed in cylindrical Plexiglas cages equipped with a system from programmed continuous infusion of either ethanol or saline solutions via catheters chronically implanted into the right external jugular vein [45]. The IV preparation used is essentially that reported by Nicolaidis *et al.* [27]. A daily 12/12 hr dark/light cycle was maintained throughout all experiments. Food and water were available ad lib except where otherwise indicated.

Determination of Metabolic Tolerance (MT)

Acute injection procedure. MT measures were obtained by determining the rate of disappearance of ethanol from the blood following the IV administration of a 1.5 g/kg dose prepared from 95% ethanol diluted in physiological saline (2.1 ml). The ethanol was infused via the intra-jugular catheter during 5 min at a constant rate of 0.42 ml/min using a motor-driven syringe. One hundred μl blood samples were collected through the catheter at 15, 60, 120, 180 and 240 min after the end of the injection. In order to prevent against contamination of the samples, the catheters were filled with viscous polyvinylpyrrolidone (PVP) solution after each sample and drained before each sample [45]. Blood ethanol concentrations were measured using an enzymatic method (NAD–ADH). The best-fit line was determined by the method of least mean squares and the slope taken as the mean blood ethanol elimination rate or MT in mg/ml/hr.

Chronic infusion procedure. This method provided a measure of MT changes during the course of chronic treatment. After an initial measure of the ethanol elimination rate using the acute injection procedure, the ethanol concentration in the blood at time zero could be extrapolated from the line of best-fit and subsequently Widmark's "*r*" value representing the volume of distribution of ethanol in relation to total body mass could be calculated ($r=A_0/C_0$; where A_0 is the quantity of ethanol injected in g/kg and C_0 is the blood ethanol concentration at time zero in g/l). Consequently, with the acute injection method the ethanol elimination rate in g/l/hr referred to as $\beta_{60}=A_0/r\cdot t'$ (where t' is the time in hours required to eliminate the total quantity of ethanol injected (A_0) from the body [11,33]. In the situation involving chronic ethanol infusion, any change in blood ethanol concentration occurring during a given time interval (t) is directly related to the quantity of ethanol administered (A_i/r) and that eliminated (β_1), i.e.: $dC/dt=Q_i-Q_0$ (where, respectively, Q_i and Q_0 represent the rate of rise and fall in ethanol concentration within the compartment). Inversely, as employed in the present study, the amount of ethanol eliminated every 24 hr (t) could be calculated on the basis of the known amounts of ethanol infused and the sampling of 24 hr BALs ($\beta_1=A_t/r-(C_t-C_0)$; or $Q_0=Q_i-dC/dt$).

Determination of Nervous Tolerance (NT)

The behavioral test used for assessing NT in the rat is based on a measure of the latency of onset of drinking requiring a standing-upright position after water-deprivation and following the injection of a challenge dose of ethanol [26]. Each NT determination extended over a 2-day period. At 10:00 a.m. on the first day (Habituation day) 20-hr water deprivated animals received an injection (IP) of 2.1 ml isotonic saline. Twenty minutes later they were placed in the testing apparatus and the latency of drinking onset was recorded. At 2:00 p.m. the rats were returned to their home cages and again water-deprived until 10:00 a.m. the next day (Test Day). At this time the testing procedure was repeated after a saline injection which now contained 1.5 g/kg of ethanol. Again drinking latency was recorded as an indication of nervous tolerance (1/NT).

Experiment 1

The purpose of this experiment was to determine the overall range of interindividual differences in MT using the acute injection procedure (N=67). Twenty-two rats representing a uniform wide distribution of MT rats (0.35–0.55 mg/ml/hr) were selected to undergo testing for NT in order to examine possible relationships between the initial degrees of tolerance manifested.

Experiment 2

The aim of this experiment was to determine MT and NT in both the naive animal and following chronic ethanol administration. The experiment extended over a 16-day period. Twelve rats received their first NT test on Days 1 and 2. Intrajugular catheters were implanted on Day 5 and the chronic treatment period was begun on Day 8 and lasted 4 days. The animals were divided into two groups; 6 rats were infused with ethanol (Group E) and 6 rats with physiological saline (Group C). The chronic treatment consisted of 6 intermittent-pulsed injections per day, at 4-hr intervals and each lasting 5 min. Animals of Group E received 1.5 g/kg of ethanol per injection contained in 2.1 ml of saline for a total daily dose of 9 g/kg. The MT measure using the acute injection procedure was performed on all rats following the first and last injection of the chronic treatment series. NT after chronic treatment was re-tested on Days 15 and 16.

Experiment 3

In this experiment the time course of MT changes was investigated throughout the period of chronic ethanol treatment (continuous infusion procedure). After implant of intra-jugular catheters an initial determination of MT was performed according to the acute injection procedure. Two days later, infusion with ethanol was started at 2:00 p.m. and 100 μl blood samples were collected through the catheter, after rinsing with PVP, at this same time every day for the duration of the chronic treatment (4–9 days). In one group of animals weighing 300–330 g (Group A, N=5), the individual

TABLE 1

EXPERIMENT 2. SUMMARY OF THE RESULTS OBTAINED REGARDING METABOLIC TOLERANCE (MT) USING THE ACUTE INJECTION PROCEDURE AND NERVOUS TOLERANCE (NT=1/LATENCY) AS MEASURED IN THE DRINKING TEST. PRE- AND POST-TREATMENT MT AND NT LEVELS AS WELL AS BODY WEIGHTS ARE ILLUSTRATED FOR BOTH ETHANOL-TREATED (GROUP E) AND SALINE-TREATED (GROUP C) ANIMALS

		Pre-Treatment	Post-Treatment	% Change	Significance (Mann-Whitney)
Ethanol Elimination Rate (mg/ml/hr)	E	0.436 ± 0.015	0.535 ± 0.019	+22.7	$p<0.01$
	C	0.459 ± 0.021	0.448 ± 0.026	- 2.4	
Drinking Latency (min)	E	117 ± 25	43 ± 20	-63.8	$p<0.05$
	C	111 ± 20	93 ± 27	-16.0	
Body Weight	E	345.0 ± 18	336.5 ± 18	- 2.4	
	C	345.5 ± 13	346.5 ± 12	+ 0.2	

doses of ethanol administered during the first 24 hr were set just above the amounts that each animal could theoretically eliminate as estimated in the preceding acute injection situation. On subsequent days (Days 1–7), all doses were progressively increased in steps of 1 g/kg/day over 2-day periods (Fig. 1). In another group weighing 398–479 g (Group B, N=6), the individual doses were adjusted regardless of initial MT levels and generally in ascending order from 7–11 g/kg/day. The length of treatment varied with each animal depending on the rate of onset of severe signs of intoxication (unconsciousness, weak respiration and heart-beat, absence of swallowing or eyeblink reflex; coma [19]). The ethanol infusion was stopped in comatose rats although in some cases the treatment was continued using a much lower dose (Fig. 2).

RESULTS

Experiment 1

Marked inter-individual differences in MT and NT were observed in the naive animal. MT as determined with the acute injection procedure varied between 0.312 and 0.570 mg/ml/hr (mean: 0.427 ± 0.006, N=67). The drinking latencies provided by the NT test ranged from 45 to 255 min (mean: 125 ± 12, N=22). No significant correlation was found between NT and corresponding MT values measured in the same animals (r′=0.14).

Experiment 2

The results for ethanol-treated (Group E) and saline-treated (Group C) rats are shown in Table 1. Differences between Group E and Group C in their modified response to ethanol were found to be significant with regard to both MT and NT. Wilcoxon *t*-tests comparing differences between pre- and posttreatment scores yielded a significant effect on both tolerances for Group E animals ($p<0.05$) whereas no significant consequences of the treatment was found for Group C.

The mean body weights recorded before chronic treatment (Day 1) were 345.0 g ± 18 for Group E and 345.5 g ± 13 for Group C. The post-treatment weights observed on the last day of the experiment (Day 16) were respectively 336.5 g ± 18 and 346.5 g ± 12 indicating an average weight loss of 8.5 g in Group E animals and a very slight gain in weight for the rats of Group C. It should be mentioned that all Group E animals showed withdrawal signs such as hyperexcitability, tremor and audiogenic convulsions [19] after the cessation of ethanol administration.

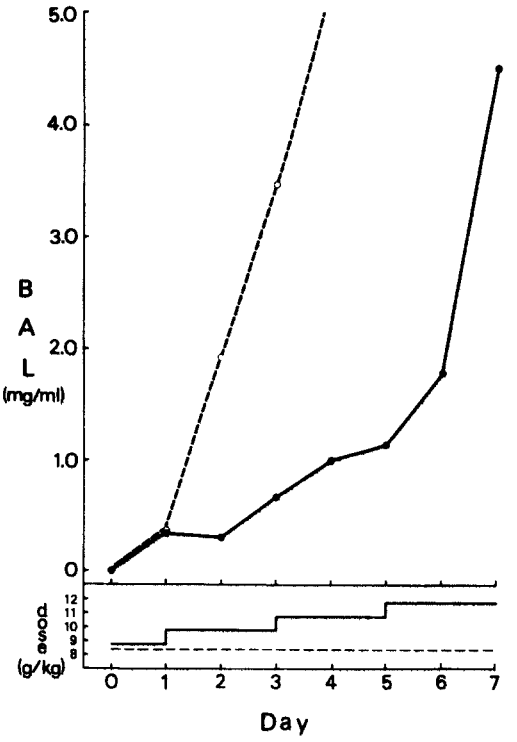

FIG. 1. Experiment 3. Group A (N=5). Graphic representation showing the mean daily blood alcohol levels (BAL) measured throughout the period of chronic ethanol treatment (solid line curve). The daily doses administered (continuous infusion procedure) ranging from 8.7 to 11.7 g/kg/day are illustrated at the bottom of the figure. Theoretical mean BALs for the first few days of chronic treatment assuming no change from initial MT levels (acute injection procedure) are represented by the broken line curve.

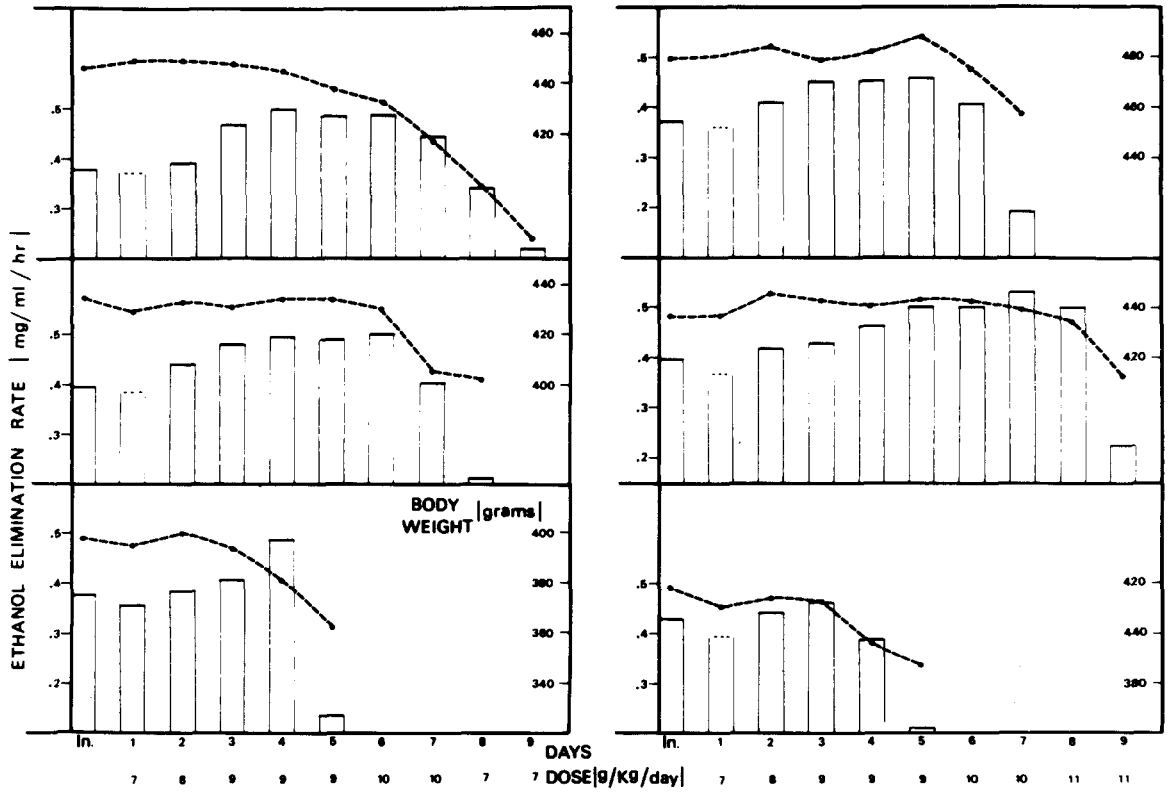

FIG. 2. Experiment 3. Group B (N=6). Individual data obtained using the chronic infusion procedure for the determination of daily MT rates (histograms); the curves show the daily body weights recorded during the chronic treatment period. Maximal MT rates averaged 25.6% above pre-treatment levels (In).

Experiment 3

The mean ethanol elimination rate as determined initially with the acute injection procedure was 0.420 ± 0.008 mg/ml/hr for the rats of Group A. Figure 1 shows the mean daily BALs measured during the chronic treatment period as well as some theoretical BALs, that is, those expected based on initial MT values and assuming no change in rate (C_t–C_0=A_t/r–β_{60}·t; see Method). The BALs were observed to rise more or less steadily up until Day 5, and subsequently exhibited a sharp increase during the final two days. At this stage BALs as high as 5.5 mg/ml were attained and all rats were comatose. The daily MT rates derived according to the continuous infusion procedure indicated an overall increase in MT as of the second day of treatment (0.495 ± 0.018 mg/ml/hr, $p<0.05$; Mann-Whitney U test) and attained a maximum of 0.568 ± 0.004 mg/ml/hr on Day 6 (35.2%, $p<0.01$). MT was then observed to decline suddenly on the last day to 0.479 ± 0.025 mg/ml/hr, a 15.7% reduction ($p<0.01$) compared to the previous day. The progressive increase in BALs were accompanied by a reduction in food and water intake. Virtually no consumption occurred on Days 6 and 7.

The individual data obtained for rats of Group B are presented in Fig. 2 where the histograms and curves show the daily estimated MT values and body weights respectively. For all animals, an increment in MT levels was observed by the second day of treatment and the maximal rates averaged 25.6% above the pre-treatment levels. The increases in MT noted during the initial stages of the treatment coincided with moderate alterations in body weight. Overall, the daily increases in MT averaged 0.026 mg/ml/hr ± 0.005 and were associated with a slight mean reduction in body weight of 0.7 ± 1 g per day. Similarly, as in Group A, MT rates decreased as the intoxication proceeded, and this was again associated with a drop in body weight. Overall daily decreases in MT averaged 0.124 mg/ml/hr ± 0.028 and coincided with a mean loss in body weight of 13.0 ± 2 g per day.

DISCUSSION

The present data demonstrate significant increases in metabolic tolerance induced by chronic ethanol treatment. Whereas such modifications were observed after 4 days of intermittent pulsed ethanol administration (Experiment 2), with the continuous infusion method (Experiment 3) increases in MT were already apparent 48 hr following the onset of treatment.

The relative increases in MT observed in Experiments 2 (22.7%) and 3 (35.2 and 25.6%) are compatible to those previously reported [24,40] employing different methods and durations of chronic administration. Nevertheless, other ethanol treatments have generated even more striking elevations in metabolic rate: about 50% [18], 52.2% [46], 54–58% [7], 68% [9] rat; >100% [5], mouse). In human subjects, MT enhancement has been observed after a period of chronic ethanol ingestion: 68% [23] and higher rates of MT: >100% [13] have been demonstrated in recently drinking alcoholics compared to controls.

It is difficult to explain the differences in the potential for MT adaptation reported. It is likely that, in addition to such factors as dose and pattern of ethanol administration

the duration of treatment is critical in the induction of MT. Consequently, saturation of the adaptive processes involved might not have been achieved within the relatively short period of chronic injections of Experiment 2. The results of Experiment 3 point to another variable, the nutritive state of the animal, which could interfere with adaptive MT processes. Although initial adaptive increases were noted, these were found to be readily reversible. Prolonging the intoxication period in all cases led to rapid drops in MT, often attaining levels far below those present prior to the treatment. Furthermore, reduced MT rates coincided with a marked decrease in food intake and body weight. This is consistent with the well-established role of nutritive factors in affecting the individual's capacity for ethanol elimination [14, 28, 29, 38, 44, 47]. In this regard, it should be noted also that the ethanol-treated animals of Experiment 2 exhibited a loss in body weight relative to controls. The nutritional differences between groups imply that, although the MT rates were significantly increased after exposure to ethanol, the values obtained may in fact be underestimated and may not be indicative of the actual potential for metabolic adaptation. Differences in both the parameters of intoxication used, as well as in the control of nutritive factors, could underlie some discrepancies in reports regarding both the direction and degree of MT changes induced by chronic ethanol administration.

The results of Experiment 2 demonstrated a significant increase in NT (63.8%) after 4 days of ethanol treatment. This phenomenon of acquired tolerance (nervous tolerance) has been examined extensively in the literature, and the reports vary widely with regard to the periods of ethanol administration employed for the development of tolerance (see [12] for review). The differences in the data may result from differences in the alcohol doses, in the particular behavioral criteria used and the species examined. In the rat, such adaptive changes have been observed following durations of ethanol exposure similar to those used in the present study [16, 19, 20], although maximal tolerance has been shown to be attained only after 2–3 weeks [12,16] using progressively increasing daily doses up to 9 g/kg. Regarding the minimum time required to observe the phenomenon Majchrowicz and Hunt [20] using repeated ethanol intubations noted a build-up in tolerance as early as the first day of treatment. However, in comparing NT data derived from studies involving such short periods of intoxication, the distinction between acute and chronic tolerance must be kept in mind. In the present study NT was retested 3 days after the termination of the chronic treatment and thus measured long term effects. In time course studies, such carry-over effects of tolerance have been observed to last 2–3 weeks following the cessation of alcohol treatment [12,16]. A better comparison of the various findings would be possible in the light of more detailed information regarding the respective time course of acquisition and loss of tolerance depending on the pattern of ethanol administration (single, repeated or intermittent, continuous) and the relationship between acute and chronic tolerance.

Signs of physical dependence were observed following 4 days of ethanol treatment (Experiment 2) which is compatible with exposure times previously reported [19,20] to produce the phenomenon. Withdrawal signs were associated with both increased (Experiment 2) and decreased (Experiment 3) levels of MT, thus suggesting no relation between modifications in this factor and the development of physical dependence. Other data, consistent with this view, were derived from experiments in which rats undergoing 1.5 day of ethanol intoxication using the intermittent pulsed-infusion schedule subsequently demonstrated an increase in MT without revealing any of the signs of physical dependence characterized by Majchrowicz [19].

A comparison of the various MT and NT values obtained indicated no relation between the two tolerance factors in the naive animal however both were enhanced following chronic ethanol treatment. This suggests that MT values measured in either the naive or previously intoxicated animal largely reflect CNS sensitivity toward ethanol. Nevertheless it would be inappropriate to disregard the MT contribution, particularly when comparing subjects exhibiting large differences in their rates of ethanol elimination such as the extremes recorded in our sampling (0.570 and 0.312 mg/ml/hr). Assuming equal degrees of "manifest" NT as determined by behavioral testing, the subject with the higher rate of ethanol elimination would in actual fact have a lower "real" NT because of the relatively lower BALs present and the shorter total duration of ethanol exposure. Consequently the effect of MT should be assessed and taken into account in correcting for manifest nervous tolerance.

An attempt to quantify the relative contribution of either tolerance factor to the adpative NT changes observed in the animals of Experiment 2 can be made by extrapolating the BALs present at the time of drinking onset in the NT test. By substituting the mean latency values provided by the NT tests performed prior to and following the chronic treatment into the corresponding linear equations for blood ethanol elimination, NT can be expressed in terms of BALs correcting for concomitant variations in MT. Accordingly, the pre- and post-treatment values obtained are respectively 89.25 and 148.88 mg/100 ml for Group E and 92.38 and 109 mg/100 ml for Group C representing mean NT increases of 66.8 and 18.2%. The similarity between the latter tolerance values and those obtained using drinking latency alone suggests that, under the present experimental conditions, the manifest NT increase is more the result of CNS adaptation than a less effective ethanol stimulus due to more rapid metabolism.

Because NT reflects the intensity of the postingestive effects of ethanol it may be linked to the defense mechanism, such as those intervening in taste aversion conditioning, which would provide protection against excessive alcohol consumption (toxicophobia). There is evidence that mouse strains which avoid drinking ethanol exhibit a higher sensitivity to the depressant central effects of the drug [10, 30, 31, 41, 42]. Thus NT would appear to constitute the critical factor regarding both the initial degree of voluntary acceptance to drink alcohol and the secondary alterations in such acceptance induced by chronic intoxication.

REFERENCES

1. Collins, A. C., M. E. Yeager and S. S. Panter. Variations in alcohol metabolism: influence of sex and age. *Pharmac. Biochem. Behav.* **3**: 973–978, 1975.
2. Eckardt, M.J. Conditioned taste aversion produced by the oral ingestion of ethanol in the rat. *Physiol. Psychol.* **3**: 317–321, 1975.
3. Eckardt, M. J., A. J. Skurdal and J. S. Brown. Conditioned taste aversion produced by low doses of alcohol. *Physiol. Psychol.* **2**: 89–92, 1974.
4. Ewing, J. A., B. A. Rouse and E. D. Pelizzari. Alcohol sensitivity and ethnic background. *Am. J. Psychiat.* **131**: 206–210, 1974.

5. Griffiths, P. J., J. M. Littleton and A. Ortiz. Changes in monoamine concentrations in mouse brain associated with ethanol dependence and withdrawal. *Br. J. Pharmac.* **50:** 489–498, 1974.
6. Hanna, J. M. Metabolic responses of Chinese, Japanese and Europeans to alcohol. Alcoholism. *Clin. exp. Res.* **2:** 89–92, 1978.
7. Hawkins, R. D., H. Kalant and J. M. Khanna. Effects of chronic intake of ethanol on rate of ethanol metabolism. *Can. J. Physiol. Pharmac.* **44:** 241–257, 1966.
8. Heston, W. D. W., V. G. Erwin, S. M. Anderson and H. Robins. A comparison of the effects of alcohol on mice slectively bred for differences in ethanol sleep-time. *Life Sci.* **14:** 365–370, 1974.
9. Israel, Y., J. M. Khanna, H. Kalant, D. J. Stewart, J. A. MacDonald, G. Rachamin, S. Wahid and H. Orrego. The spontaneously hypertensive rat as a model for studies of metabolic tolerance to ethanol. Alcoholism. *Clin. exp. Res.* **1:** 39–42, 1977.
10. Kakihana, R., D. R. Brown, G. E. McClearn and I. R. Tabershaw. Brain sensitivity to alcohol in inbred mouse strains. *Science* **154:** 1574–1575, 1966.
11. Kalant, H. Absorption, diffusion, distribution and elimination of ethanol; effects on biological membranes. In: *The Biology of Alcoholism*, edited by B. Kissin and H. Begleiter. New York: Plenum Press, 1972, Vol. 1, Biochemistry, pp. 1–62.
12. Kalant, H., A. E. Le Blanc and R. J. Gibbins. Tolerance to, and dependence on, ethanol. In: *Biological Basis of Alcoholism*, edited by Y. Israel and J. Mardones. Wiley-Interscience, 1971, pp. 235–269.
13. Kater, R. M. H., N. Carulli and F. L. Iber. Differences in the rate of ethanol metabolism in recently drinking alcoholic and non-drinking subjects. *Am. J. clin. Nutr.* **22:** 1608, 1969.
14. Kinard, E. W., M. G. Hay and G. H. Nelson. Effects of starvation on alcohol metabolism and hepatic enzyme activities in dogs. *Q. Jl Stud. Alc.* **21:** 203–207, 1969.
15. LeBlanc, A. E., R. J. Gibbins and H. Kalant. Behavioral augmentation of tolerance to ethanol in the rat. *Psychopharmacology* **30:** 117–122, 1973.
16. LeBlanc, A. E., H. Kalant and R. J. Gibbins. Acquisition and loss of tolerance to ethanol by the rat. *J. Pharmac. exp. Ther.* **168:** 244–250, 1969.
17. Lester, D., M. Nachman and J. LeMagnen. Aversive conditioning by ethanol in the rat. *Q. Jl Stud. Alc.* **31:** 578–586, 1970.
18. Lieber, C. S. and L. M. DeCarli. The role of the hepatic microsomal oxidizing system (MEOS) for ethanol metabolism in vivo. *J. Pharmac. exp. Ther.* **181:** 279, 1972.
19. Majchrowicz, E. Induction of physical dependence upon ethanol and the associated behavioral changes in rats. *Psychopharmacology* **43:** 245–254, 1975.
20. Majchrowicz, E. and W. A. Hunt. Ethanol tolerance and physical dependence in rats. *Psychopharmacology* **50:** 107–112, 1976.
21. Majchrowicz, E., M. A. Lipton, J. L. Meek and L. Hall. Effects of chronic ethanol consumption on the clearance of acutely administered ethanol and acetaldehyde from blood in rats. *Q. Jl Stud. Alc.* **29:** 553–557, 1968.
22. Mendelson, J. H. Ethanol-1-^{14}C metabolism in alcoholics and nonalcoholics. *Science* **159:** 319–320, 1968.
23. Mendelson, J. H. Ethanol metabolism in alcoholics. In: *Biological Aspects of Alcohol Consumption*, edited by O. Forsander and K. Eriksson. Finnish Foundation, 1971, **20:** 25–31.
24. Mezey, E. Duration of the enhanced activity of the microsomal ethanol-oxidizing enzyme system and rate of ethanol degradation in ethanol-fed rats after withdrawal. *Biochem. Pharmac.* **21:** 137–142, 1972.
25. Mezey, E. and F. Tobon. Rates of ethanol clearance and activities of the ethanol-oxidizing enzymes in chronic alcohol patients. *Gastroenterology* **61:** 707–715, 1971.
26. Miceli, D., J. LeMagnen and H. Katz. A simple drinking test for measuring the effects of ethanol on the CNS. In preparation.
27. Nicolaidis, S., N. Rowland, M. J. Meile, P. Marfaing-Jallat and A. Pesez. A flexible technique for long term infusions in unrestrained rats. *Pharmac. Biochem. Behav.* **2:** 131–136, 1974.
28. Owens, A. H. and E. K. Marshall. The metabolism of ethyl alcohol in the rat. *J. Pharmac. exp. Ther.* **115:** 360–370, 1955.
29. Pawan, G. L. S. Effect of the antecedent diet on the rate of metabolism of alcohol (ethanol) in man. *Proc. Nutr. Soc.* **27:** 58A–59A, 1968.
30. Randall, C. L., J. A. Carpenter, D. Lester and H. J. Friedman. Ethanol-induced mouse strain differences in locomotor activity. *Pharmac. Biochem. Behav.* **3:** 533–535, 1975.
31. Randall, C. L. and D. Lester. Differential effects of ethanol and pentobarbital on sleep time in C57BL and BALB mice. *J. Pharmac. exp. Ther.* **188:** 27–33, 1974.
32. Reed, E. T. Racial comparisons of alcohol metabolism: Background, problems, and results. Alcoholism. *Clin. exp. Res.* **2:** 83–87, 1978.
33. Reed, E. T. and H.Kalant. Bias in calculated rate of alcohol metabolism due to variation in relative amounts of adipose tissue. *J. Stud. Alc.* **9:** 1773–1776, 1977.
34. Riley, E. P., E. X. Freed and D. Lester. Selective breeding of rats for differences in reactivity to alcohol: an approach to an animal model of alcholism. I. General procedures. *J. Stud. Alc.* **37:** 1535–1547, 1976.
35. Riley, E. P., E. D. Worsham, D. Lester and E. X. Freed. Selective breeding of rats for differences in reactivity to alcohol. An approach to an animal model of alcoholism. II. Behavioral measures. *J. Stud. Alc.* **38:** 1705–1717, 1977.
36. Rodgers, D. A., G. E. McClearn, E. L. Bennett and M. Hebert. Alcohol preference as a function of its caloric utility in mice. *J. comp. physiol. Psychol.* **56:** 666–672, 1963.
37. Rozin, P. and J. W. Kalat. Specific hungers and poison avoidance as adaptive specialization of learning. *Psychol. Rev.* **78:** 459–486, 1971.
38. Samson, H. H., D. C. Morgan, C. M. Price, M. Tang and J. L. Falk. Ethanol elimination rates in normal and ethanol dependent animals. *Pharmac. Biochem. Behav.* **5:** 335–341, 1976.
39. Schaefer, J. M. Alcohol metabolism and sensitivity reaction among the Reddis of South India. Alcoholism. *Clin. exp. Res.* **2:** 61–69, 1978.
40. Schlesinger, K., E. L. Bennett and M. Hebert. Effects of genotype on prior consumption of alcohol on rates of ethanol -1-^{14}C metabolism in mice. *Q. Jl Stud. Alc.* **28:** 231–235, 1967.
41. Schneider, C. W., S. K. Evans, M. B. Chenoweth and F. L. Beman. Ethanol preference and behavioral tolerance in mice, biochemical and neurophysiological mechanisms. *J. comp. physiol. Psychol.* **82:** 466–474, 1973.
42. Schneider, C. W., P. Trzil and R. D'Andrea. Neural tolerance in high and low ethanol selecting mouse strains. *Pharmac. Biochem. Behav.* **2:** 549–552, 1974.
43. Sheppard, J. R., P. Albersheim and G. E. McClearn. Enzyme activities and ethanol preference in mice. *Biochem. Genet.* **2:** 205–212, 1968.
44. Smith, M. E. and H. W. Newman. The rate of ethanol metabolism in fed and fasting animals. *J. biol. Chem.* **234:** 1544–1549, 1959.
45. Steffens, A. B. A method for frequent sampling of blood and continuous infusion of fluids in the rat without disturbing the animal. *Physiol. Behav.* **4:** 833–836, 1969.
46. Videla, L., J. Bernstein and Y. Israel. Metabolic alterations produced in the liver by chronic ethanol administration: increased oxidative capacity. *Biochem. J.* **134:** 507–514, 1973.
47. Vitale, J. J., J. Nay and D. M. Hegsted. Partial starvation and alcohol metabolism: an example of adaptation to undernutrition. *J. Nutr.* **53:** 533–541, 1954.