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Ethanol Dependence Produced in Rats by Nutritionally Complete Diets

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GOLDMAN, M. E., S. S. MILLER, R. L. SHOREY AND C. K. ERICKSON. *Ethanol dependence produced in rats by nutritionally complete diets.* PHARMAC. BIOCHEM. BEHAV. 12(4) 503–507, 1980.—Nutritionally complete diets formulated according to American Institute of Nutrition guidelines were used to make rats dependent upon ethanol. When intubated with a diet-ethanol solution for four days rats maintained initial body weight. When forced to consume the solution as the sole source of nutrients and water for nineteen days, rats gained weight. All animals developed severe withdrawal signs as measured by the intensity of tremors and spastic rigidity. The diet ingredients did not alter the absorption of the ethanol. The results demonstrate that physical dependence on ethanol can be induced in the rat without nutritional impairment.

Ethanol Nutritionally complete liquid diets Physical dependence Withdrawal

MUCH of the experimentation on the pathogenesis of alcoholism utilizes laboratory animals. The plethora of animal models of physical dependence and alcoholism in the last decade has recently been reviewed by Goldstein [6].

Most existing rat models of ethanol physical dependence have limitations for certain applications. For example, intragastric intubation methods require the administration of large doses of ethanol diluted in water and produce constant intoxication that inhibits the ability of the rat to eat, resulting in severe weight loss [12]. Dietary regimens containing high concentrations of fat such as that of De Carli and Lieber [4] are used primarily for inducing fatty liver but also allow the development of physical dependence. Commercial liquid diets for human consumption, e.g. Metrecal and Sustacal [1,19] may not supply nutritional requirements of the rat as established by the American Institute of Nutrition (AIN) [17]. These diets also contain flavorings and preservatives which could have pharmacological or toxicological effects.

Studies on the effect of nutrition on the symptomatology of ethanol withdrawal have yielded conflicting results. Many models of dependence require restricted food intake and weight reduction in the experimental paradigm [2,3]. Ogata *et al.* [15] found that when body weight was severely depleted during the production of ethanol dependence, mice manifested greater withdrawal signs compared to animals whose weights were not greatly depleted. Baker *et al.* [1] similarly reported that nourished rats were able to tolerate greater doses of ethanol than nutrient-deprived rats and that the severity of withdrawal was less in the nourished rats. In contrast, Wallgren *et al.* [20] and Lieber and De Carli [10] found that rats intubated with a liquid diet containing ethanol, or forced to consume an ethanol liquid diet, developed severe withdrawal reactions.

The purpose of this study was to determine if rats receiving nutritionally complete liquid diets could be rendered dependent upon ethanol as demonstrated by severe withdrawal signs. The liquid diet containing ethanol used in this study prevented the weight loss associated with the method of Majchrowicz [12] but still allowed the expression of the withdrawal syndrome. In addition, when the liquid diet containing ethanol was offered as the sole source of nutrients and fluid, rats gained weight and became dependent after 19 days.

METHOD

Animals and Diet

Two methods for producing dependence on ethanol were compared in this study. The short-term method was similar to that of Majchrowicz [12] and lasted four days. The long-term method involved a 19-day exposure to a liquid diet-ethanol mixture as the sole source of food and water. In the short-term method, Sprague-Dawley rats (Mid-Continent Research, Shawnee, KS, 200–250 g, 12 hour light/dark cycle housed in groups of 3–5) were orally intubated with 10% (w/w) ethanol in water (water-ethanol) or in liquid diet (diet-ethanol). The composition of the diet-ethanol solution used for intubation is shown in Table 1 and is a modification of a diet originally published by Shorey *et al.* [18]. This diet

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TABLE 1

COMPOSITION OF LIQUID DIETS CONTAINING ETHANOL (ETOH) AT VARIOUS PERCENTAGES OF TOTAL ENERGY

Component (g/kg of total diet)	0% ETOH	20 % ETOH	30% ETOH	35% ETOH	40% ETOH
Micropulverized casein†	42.0	42.0	42.0	42.0	42.0
L-methionine†	0.6	0.6	0.6	0.6	0.6
Corn oil (Mazola)‡	10.5	10.5	10.5	10.5	10.5
AIN vitamin mix†	2.1	2.1	2.1	2.1	2.1
AIN mineral mix†	7.3	7.3	7.3	7.3	7.3
Sucrose‡	25.0	25.0	25.0	25.0	25.0
White dextrin†	110.0	70.0	50.0	40.0	30.0
Sodium carrageenate§ (Viscarin)	3.0	5.0	5.0	5.0	0.25
Choline bitartrate†	0.4	0.4	0.4	0.4	0.4
95% ethanol‡	—	24.4	36.5	42.6	48.7
Deionized water	799.1	812.7	820.6	824.5	295.4*

Diets were prepared according to the method of Shorey *et al.* [18].

*Less water was used in the preparation of this diet-ethanol solution to make it analogous to the 10% (w/w) water-ethanol solution.

†Obtained from ICN Pharmaceuticals Inc., Cleveland, OH.

‡Obtained locally.

§Obtained from Marine Colloids, Rockland, ME.

was prepared according to AIN guidelines [17] and contains recommended quantities of all nutrients, vitamins and minerals. This diet includes 40% of energy as ethanol which was isoenergetically substituted for white dextrin. Since this diet is more energy dense than the standard Shorey *et al.* [18] diets, rats were given access to water ad lib. Intubations were carried out as described by Majchrowicz [12]. Before each intubation, every animal was rated for the severity of intoxication and given a dose of the diet-ethanol or water-ethanol solutions based upon the degree of intoxication. Rats that were rated normal received a 5 g/kg dose of ethanol. Rats were intubated at 0700, 1200, 1700 and 2400 for four days. Control animals were intubated with a liquid diet solution containing white dextrin isoenergetically substituted for ethanol (0% ETOH, Table 1). The liquid diet given to control animals was equivalent to the average volume given to the diet-ethanol group at the previous intubation period.

For the long-term method, Sprague-Dawley rats weighing 100–120 g (42–49 days old) were first placed in individual wire bottom stainless steel cages and maintained on stock lab chow (Ralston Purina Co., St. Louis, MO) and water ad lib until they attained an average weight of 235 g. The animals were then divided into 3 groups. Rats in Group 1 received ethanol in a concentration equal to 20% of total energy on Days 1–3 (Table 1). On Days 4–6, the ethanol concentration was increased to 30% of total calories. On Day 7 and thereafter the ethanol concentration was equal to 35% of total energy. Groups 2 and 3 were both fed the control diet with no ethanol. Group 2 was pair-fed to the mean intake of Group 1, and Group 3 received the diet ad lib.

Withdrawal

In order to more closely compare the onset and duration of the withdrawal syndrome in the short-term versus the long-term dependence methods, all rats received an intragastric intubation of ethanol at 2200 on the final night of the dependence production phase of the experiment. At 2200 on Day 19 animals in Group 1 of the long-term study received an intubation of the diet-ethanol solution in amounts varying with the degree of intoxication. Non-intoxicated animals received a 6 g/kg dose of ethanol. To insure that rats made dependent by the short-term method were not overdosed, no animals in this group were ever given more than 5 g/kg ethanol at any intubation period including the final intubation at 2200 on Day 4.

In all experiments, withdrawal was quantitated by a modification of the method of Majchrowicz [12]. Instead of giving scores above 0 only to withdrawing animals, this scale rates the signs that are present both in normal animals to a small extent and in withdrawing animals to a much greater extent but absent or very low in intoxicated animals. General rigidity, general tremors, tail tremors and caudal tremors were each rated 0–3 depending upon severity. Heavily intoxicated animals received a score of 0 for each sign since they did not display any muscular rigidity or tremors. Animals that were only slightly intoxicated received a score of 1 for some signs. Normal animals generally had a small amount of muscular tension yielding a score of 1–2 for each characteristic and a total tremor-rigidity score of 4–6. Animals with scores of at least 8 were considered to be in withdrawal (2–3 for each characteristic). Withdrawing animals typically had the following signs: spastic body posture and movements due to the extreme rigidity, prominent tremors of the entire body when grasped (general tremors), stiff tail with significant tremors and a shaking caudal region. The maximal withdrawal score was 12.

Ethanol Absorption

To determine if the liquid diet solution altered the absorption of ethanol from the gastrointestinal tract, 10 rats, food-deprived overnight, were orally intubated with 5 g/kg ethanol in a 10% w/v solution with either water or diet (40%

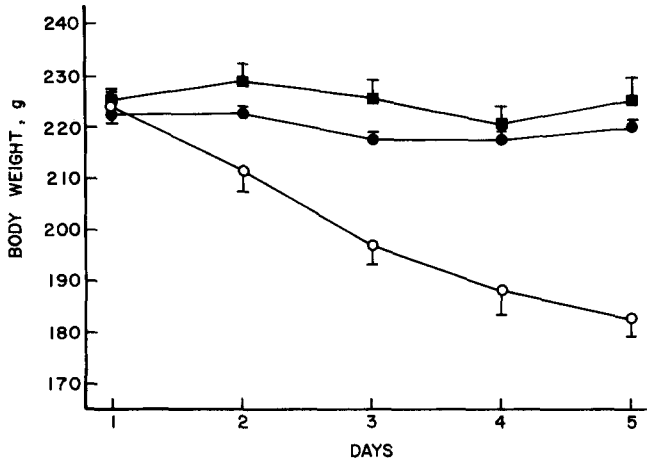

FIG. 1. Body weights in the short-term dependence experiment. (■) Group 1, diet-ethanol group, (N=13); (○) Group 2, water-ethanol group (N=8); (●) Group 3, control-diet group (N=5). Group means were significantly different (P=0.024 on Day 4 and $p=0.002$ on Day 5) by one-way analysis of variance. Pair-wise multiple comparison of means (Neuman-Keuls technique, Hartly modification) demonstrated that the means for Group 2 were significantly different at the 0.05 level from Groups 1 and 3 which were not different from each other.

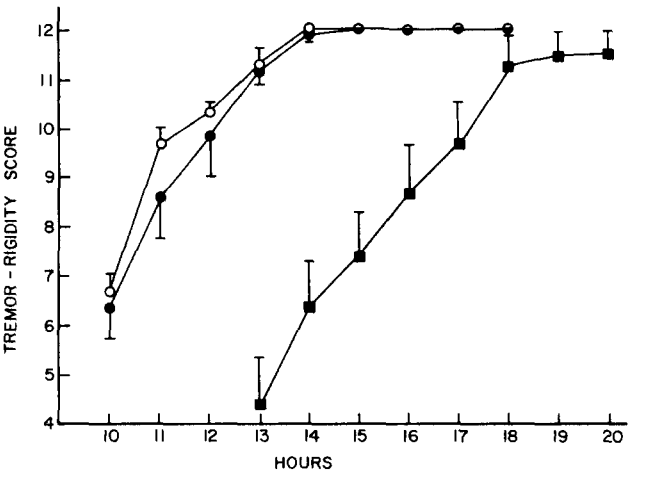

FIG. 3. Tremor-rigidity scores of dependent rats. (●) diet ethanol short-term group (N=13); (○) water ethanol short-term group (N=8); (■) diet-ethanol long-term group (N=11).

of total energy). Blood samples (50 μl) were taken by retroorbital puncture 1, 2, 3, 4, 8, 16 and 24 hours after intubation and analyzed for ethanol with a Perkin-Elmer F-40 gas chromatograph by the micromethod of LeBlanc [9] modified as reported by Erickson [5].

RESULTS

In the short-term experiment the average total daily dose of ethanol was 11.8 ± 0.5 g/kg (mean ± SE) for the water-ethanol group and 11.9 ± 0.6 g/kg for the diet-ethanol group. On Day 1 of the long-term experiment the mean ethanol consumption was 6.7 ± 0.3 g/kg. The daily ethanol consumption increased linearly to 10.9 ± 0.6 g/kg on Day 14 and then showed no significant change for the remainder of the study.

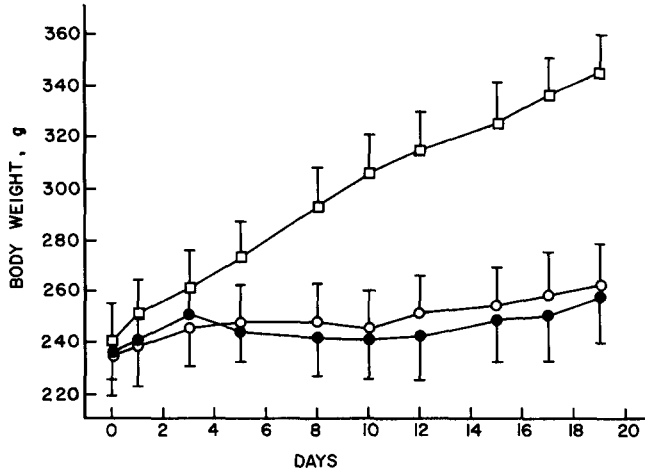

FIG. 2. Body weights in the long-term dependence experiment. (●) Group 1, diet-ethanol group (N=11); (○) Group 2, pair-fed control group (N=10); (□) Group 3, ad lib control group (N=10). Final body weights of Group 3 were significantly different from the other two groups at the 0.05 level.

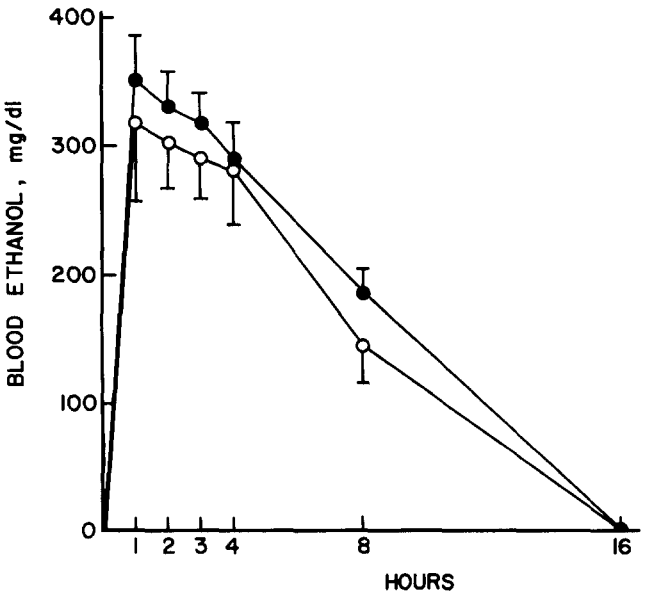

FIG. 4. Blood ethanol levels after an acute dose of 5.0 g/kg ethanol dissolved either in diet (●) or water (○). N=5 in each group. Group means were not significantly different by one-way analysis of variance.

In the short-term method, rats intubated with diet-ethanol solution lost 1.0 ± 0.1% (mean ± SE) of initial body weight by Day 5 whereas animals receiving the water-ethanol solution lost 16.1 ± 3.9% of initial body weight (Fig. 1). Control animals intubated with the 0% ethanol-diet solution were the same weight on Day 5 as on Day 1.

In the long-term experiment, rats that consumed the liquid diet -ethanol solution for 19 days gained 9.5 ± 0.2% and pair-fed controls gained 11.5 ± 0.1% of initial body weight (Fig. 2). Rats having free access to the control diet gained 48.6 ± 0.4% of initial body weight. Thus rats consuming the liquid diet-ethanol solution gained one fourth as much as the

ad lib control group. The weights of ethanol and pair fed groups were not significantly different.

Animals in the long-term group were given a larger dose of ethanol on the final night of the dependence phase than animals in the short-term dependence group because evidence from our laboratory has shown that rats exposed to ethanol for 19 days develop significant tolerance to ethanol's depressant effects as quantitated by treadmill performance (Miller *et al.*, [14]). Figure 3 demonstrates that although the times of onset of withdrawal differed between the short-term and long-term methods all animals displayed maximal withdrawal signs that lasted at least 3 hours. No differences were observed in the signs of withdrawal between the short-term versus long-term methods or the diet-ethanol versus water-ethanol groups.

Figure 4 compares blood ethanol levels resulting from the oral administration of ethanol diluted in diet or water. The diet solution did not significantly alter the rate of absorption of ethanol from the gastrointestinal tract.

DISCUSSION

The results of the short-term study demonstrate that rats intubated with the diet-ethanol solution lose an insignificant amount of body weight whereas rats receiving ethanol-water solution lose a considerable amount of weight. Cannon *et al.* [3] and Majchrowicz [12] also found that rats intubated with a water-ethanol solution lost significant amounts of weight. Baker *et al.* [1] similarly found that rats intubated with a diet-ethanol solution had a less severe weight loss than rats intubated with a water-ethanol solution.

The results of the long-term dependence method agree with previous results from one of our laboratories (Shorey *et al.* [18]) which showed that rats receiving ethanol in a liquid diet gained weight less rapidly than ad lib controls. Similar results have also been described by other laboratories (Lieber and DeCarli, [10]).

All animals receiving ethanol developed severe withdrawal signs similar to those seen by Majchrowicz [12]. Since rats in the long-term dependence group received a larger final dose of ethanol at 2200 than those in the short-term dependence group, the onset of withdrawal signs was delayed; however, the rats displayed withdrawal signs of comparable intensity. These results are in agreement with the studies of Wallgren *et al.* [20], Pohorecky [16] and Hunter *et al.* [7].

In contrast to the results of the present study, Baker *et al.* [1] found that rats receiving a liquid diet and ethanol did not have as severe withdrawal signs as nutrient-deprived rats unless they received more ethanol each day than nutrient-deprived rats. In that study, rats were intubated for three days with Sustacal plus ethanol and withdrawal was quantitated by audiogenic seizures. Majchrowicz and Hunt [13] have shown, however, that maximal withdrawal does not occur until after four days of administration of ethanol. In addition, audiogenic seizures may not be an extremely specific or sensitive indicator of severity of withdrawal when used as the sole determinant of withdrawal. We have found that audiogenic seizures can be produced in non-dependent rats, and conversely, we have observed that rats displaying maximal withdrawal signs according to the method of Majchrowicz [12] do not always develop seizures in response to an auditory stimulus.

The results of the present study correlate well with clinical experiments which have shown that subjects having free access to ethanol and maintained on nutritionally adequate diets can display severe withdrawal symptoms, including convulsions and delerium tremens (Isbell *et al.* [8]).

Some investigators may be concerned that different methods for producing dependence on ethanol in rodents may cause different types of dependence, either qualitatively or quantitatively. In the present study, there were no apparent differences in withdrawal of animals from short-term or long-term methods, suggesting that the central brain regions responsible for expressing the withdrawal syndrome are similarly affected after different durations of chronic exposure to ethanol.

The use of a nutritionally inadequate diet during the production of dependence parallels the alcoholism syndrome where inadequate nutrition and decreased absorption lead to severe malnutrition. The primary goal of animal studies, however, should be to first determine the biochemical and pathological changes induced by chronic consumption of ethanol uncomplicated by non-specific effects such as malnutrition or other toxic compounds in the diet. For example, malnutrition may alter ethanol metabolism. It has been demonstrated by Lumeng *et al.* [11] that fasted rats have decreased ethanol elimination rates and decreased liver alcohol dehydrogenase activity compared to animals having free access to food. In addition, the synthesis of certain neurotransmitters is intimately related to the nutritional status of the animal [21]. Therefore, if the diet is deficient in choline or tryptophan, changes in cholinergic or serotonergic systems in dependent rats may be mistaken for effects of ethanol rather than the combined effects of malnutrition and ethanol.

In conclusion, it has been demonstrated that physical dependence on ethanol can be produced in rodents without nutritional compromise. This has been accomplished by employing a diet that corresponds to the current requirements of the American Institute of Nutrition [17]. (Although the present results are based upon use of the custom-formulated Shorey diet, a similar diet is now available from Bioserv, Inc., Frenchtown, NJ).

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