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Ethanol Dependence in the Rat: A Parametric Analysis[1,2]

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HUNTER, B. E., J. N. RILEY, D. W. WALKER AND G. FREUND. *Ethanol dependence in the rat: A parametric analysis.* PHARMAC. BIOCHEM. BEHAV. 3(4) 619–629, 1975. – Rats were maintained on liquid diets as their sole source of calories and fluid for 10, 15, 20, and 30 days. The diets consisted of 35–40% of total calories in the form of ethanol. This procedure resulted in substantial ethanol intake leading to behavioral intoxication. Blood ethanol concentrations were found to be elevated throughout the day with a peak during the dark phase of the light cycle. The removal of ethanol resulted in evidence of physiological dependence, including behavioral manifestation of autonomic and somatic dysfunction and an increased susceptibility to audiogenic convulsions. Ten days of ethanol exposure was found to be sufficient for the reliable induction of ethanol dependence. Further increases in ethanol exposure resulted in increased hyperexcitability as measured by susceptibility to audiogenic convulsions. The severity of withdrawal behavior was found to be correlated with the blood ethanol concentration measured upon ethanol removal. A behavioral rating scale for the evaluation of alcohol withdrawal intensity in rats is described.

Ethanol Convulsions Alcohol Ethanol dependence Physiological dependence Withdrawal syndrome

A NUMBER of methods for the induction of physiological dependence on ethanol in laboratory animals have been developed within the past decade [30]. While providing little information pertaining to those conditions necessary in the initial development of excessive alcohol consumption, these techniques have provided animal models useful in the delineation of the biological and behavioral consequences of the development of ethanol dependence. Several strategies have been utilized in the induction of physiological dependence, including the administration of ethanol orally [8, 10, 18, 25, 33], intravenously [3,43], intragastrically [4, 6, 39], or by inhalation [15,16]. Each of these divergent methodologies has in common the forced administration of ethanol, a feature that has led Mello [30] to characterize them as pharmacological models of alcohol addiction.

While several species were originally shown to develop signs of ethanol dependence similar to those reported in man [42], including dogs [6], monkeys [3, 4, 43], and mice [10, 16, 33], preliminary attempts to replicate these effects in rats proved more difficult. The rat would appear to be a particularly suitable species for use in studies involving the delineation of the neurobiological concomitants of ethanol dependence. Preliminary experiments involving the examination of electrographic activity from various brain regions [18], as well as studies of neurochemical alterations [34] during alcohol withdrawal, require large groups, practical only with small laboratory animals. The rat has several advantages over the mouse, including larger brain size, more comprehensive and detailed stereotaxic brain atlases, and a relatively greater background literature of behavioral, neuroanatomical, neurophysiological and neuropharmacological information.

A more careful application and adaptation of some basic principles derived from previous studies has led to a number

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of reports of ethanol dependence in rats within the past few years [1, 7, 8, 18, 25, 39]. These studies have been characterized either by small numbers of animals, incomplete descriptions of withdrawal symptoms, or marked differences in the duration of ethanol exposure. Recently we reported the successful adaptation [18] to the rat of the liquid diet technique previously developed by Freund for mice [10]. In this study [18] rats were maintained for 15 days on liquid diets that contained 35–40% of the calories in the form of ethanol. Removal of ethanol resulted in the time-dependent appearance of a variety of withdrawal symptoms, including tail signs, tremors, and audiogenic convulsions.

The present experiment was undertaken to examine more carefully some parameters of ethanol dependence in rats. Specificially, the experiment sought to (1) examine the relationship between duration of ethanol exposure and intensity of the ensuing withdrawal signs and (2) provide a more detailed description of the time course and symptomatology of alcohol withdrawal in the rat.

METHOD

Animals

Forty-eight male Long-Evans hooded rats, approximately 60 days old, weighing approximately 130–150 g, were used. The rats were housed individually in stainless steel cages in a colony room with a 7:00 a.m. to 7:00 p.m. light cycle.

Liquid Diets

Details of the preparation, composition, and nutritional adequacy of the liquid diets used in this experiment have been presented in detail previously [36]. The diets were prepared from a 63.3% (v/v) stock solution (prepared from 95% ethanol and distilled water) mixed with Metrecal Shape (Mead Johnson, Co.). The diets contained 35–40% of the calories in the form of ethanol – an ethanol concentration of 8.1–9.7% (v/v). The diets provided approximately 1.3 kCal/ml. Control diets were prepared in an identical fashion, except sucrose was isocalorically substituted for ethanol. The diets were fortified with Vitamin Diet Fortification Mixture, 0.3 g/100 ml of diet, and Salt Mixture XIV, 0.5 g/100 ml of diet (Nutritional Biochemicals Co.). The diets were prepared fresh daily and administered in calibrated bottles.

Procedure

The rats were divided into 4 groups (n = 10), matched as closely as possible for body weight, and were then reduced to 75% of their free-feeding weight by restricting food consumption to 5 g of pelleted laboratory food per day for 7–8 days. Each group then received liquid diets containing ethanol as their sole source of calories and fluid for 10, 15, 20, or 30 days. The initiation of food restriction and ethanol exposure was staggered, so that only two rats would be withdrawn each day to allow detailed observation. The percentage of calories in the form of ethanol was gradually increased from 35–40% by increases of one percentage point at intervals of 5 days. Blood samples (30–50 μl) from the tail were collected in heparinized capillary tubes and were frozen until subsequent determination of ethanol concentration by a gas chromatographic procedure described previously [9]. Samples were taken after 24 hr, and 5, 10, 15, 20, 25, and 30 days of ethanol exposure.

A separate group of 8 rats was used in the determination of diurnal variations in blood ethanol level. The rats were treated in a similar fashion to those described above and blood samples were collected at 2:00 a.m., 8:00 a.m., 2:00 p.m., and 8:00 p.m. To avoid possible confounding effects of blood sample collection on ethanol consumption, the 8:00 a.m. and 8:00 p.m. samples were collected on Day 14 and the 2:00 a.m. and 2:00 p.m. samples collected on Day 16 of ethanol exposure.

On the day of withdrawal the ethanol diets were removed at 8:00 a.m. Each rat was placed in an observation chamber (12 X 18 X 12 in.) that had one plexiglas side. Tap water and a liquid diet containing sucrose isocalorically substituted for ethanol were available at all times during withdrawal. Blood samples were collected at hourly intervals for at least 6 hr postwithdrawal (PW).

Each rat was carefully observed for behavioral signs of alcohol withdrawal for 8–10 hr. At 8 hr PW the susceptibility to audiogenic convulsions was assessed. The audiogenic stimulus consisted of a jangling of keys near the top of the observation chamber for a maximum of 15 sec.

Behavioral activity was also assessed during the 8 hr observation period. The floor of the observation chamber was divided into 6 squares (6 X 6 in.). Four types of behavior were scored and used to determine the level of behavioral activity. Counts were made of the number of grid crosses, rotational movements (within a square) that did not result in a grid cross, rearings against the side of the observation chamber, and periods of grooming with a duration of 5 sec. A composite activity score was derived from the sum of the total counts obtained for each behavior. To avoid possible contributions of blood sample collection to the activity score, counts were discontinued for 15 min after each hourly blood sample.

It is important to note that sucrose control groups were not included in the present experiment. The normal procedure using the liquid diet technique provides for a control group, which is pair-fed identical diets with sucrose isocalorically substituted for ethanol. However, we have previously shown that procedures similar to those used in the present experiment resulted in neither the appearance of withdrawal symptoms nor a reduction in the threshold for audiogenic convulsions in sucrose control rats [18,19]. In addition, investigators in two other laboratories have found similar results using a liquid diet procedure [1,25]. Furthermore, we have shown that susceptibility to audiogenic convulsions develops as a function of alcohol removal: rats continuing to consume equal daily quantities of ethanol, but not being withdrawn, did not have audiogenic convulsions [18]. These numerous reports led us to eliminate this control in the present experiment.

RESULTS AND DISCUSSION

The mean free-feeding weights of the 10-day (10D), 15-day (15D), 20-day (20D), and 30-day (30D) groups were respectively 264.1 g, 254.1 g, 258.6 g, and 263.3 g. The rats gained weight throughout the liquid diet treatment period and exhibited no evidence of malnutrition. As shown in Fig. 1, the rats reattained predeprivation weight after approximately 20 days of liquid diet consumption. Mean daily ethanol consumption, calculated over all groups, was 15.3 g/kg. Daily observations revealed signs of gross intoxi-

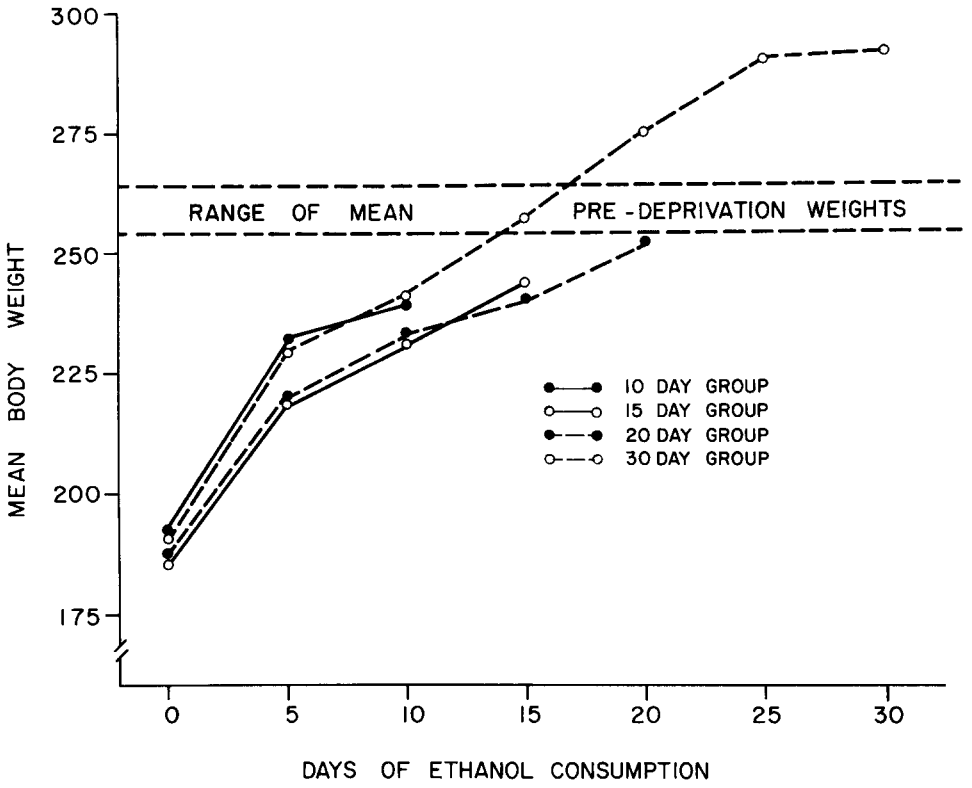

FIG. 1. Mean body weight for each group during consumption of ethanol-containing liquid diets.

cation, including docility, ataxia, and loss of motor coordination.

Patterns of Ethanol Consumption

An interesting feature of the alcoholization period was a marked cyclicity of ethanol consumption. Generally, the level of ethanol consumption was stable, ranging from 12–25 g/kg/day. However, spontaneous abstinence periods were observed, characterized by a marked decrease in ethanol consumption together with the appearance of withdrawal symptoms. Representative examples of some individual ethanol consumption patterns of rats from the 10D, 15D, 20D, and 30 D groups are shown in Fig. 2. When ethanol consumption decreased to less than 8 g/kg (indicated by dotted line), the rats exhibited signs of ethanol dependence, including tail stiffening, tremors, broad-based gait, and hyperreactivity. A spontaneous abstinence period never extended beyond 24 hr, as indicated by many of the rats shown in Fig. 2. This phenomenon, while pervasive, did not occur in all rats. Both 10A and 15E, for example, exhibited stable patterns of consumption throughout the duration of ethanol exposure. However, as the duration of ethanol exposure increased, the probability of occurrence of a spontaneous abstinence period also increased. For example, the percentage of rats exhibiting spontaneous withdrawal episodes was 40% in the 10D group, but increased to 80% in both the 20D and 30D groups. Although spontaneous abstinence appears to occur (Fig. 2) in different rats on the same calendar date, recall that the experiment was staggered. Thus, the abscissa does not represent calendar date but days of exposure.

The frequency of spontaneous withdrawal episodes was further examined by an analysis of the periodicity of ethanol consumption. The results of this analysis are shown in Fig. 3. The number of consecutive days of ethanol consumption without an abstinence period was determined and compiled into a frequency distribution. An abstinence period was defined as any day in which ethanol consumption decreased below 8 g/kg. Because scheduled withdrawal periods imposed an effective ceiling on the duration of ethanol exposure, the data were analyzed either by ignoring (Fig. 3A) or by including (Fig. 3B) those periods of ethanol consumption affected by the ceiling. For example, 30G (shown in Fig. 2) exhibited abstinence periods after 13 and 22 days of ethanol exposure. Thus, 30G was considered to have had 3 discrete periods of ethanol consumption – 12, 8, and 8 days. The 12- and 8-day periods are both included as data points in Fig. 3A. Since the final 8 days of consumption were bounded by the scheduled withdrawal period (30 days), it was not included in Fig. 3A. However, all 3 periods were included in Fig. 3B.

An examination of Fig. 3 reveals a marked periodicity of ethanol consumption at 7–12 days. In other words, rats tended to consume at least 8 g/kg/day of ethanol in periods lasting 7–12 days. It should be emphasized that the data in Fig. 3 are biased, since relatively fewer animals existed as the duration of ethanol exposure increased. However, the results indicate that the probability of a spontaneous abstinence period increases markedly as the duration of ethanol exposure reached 7–12 days.

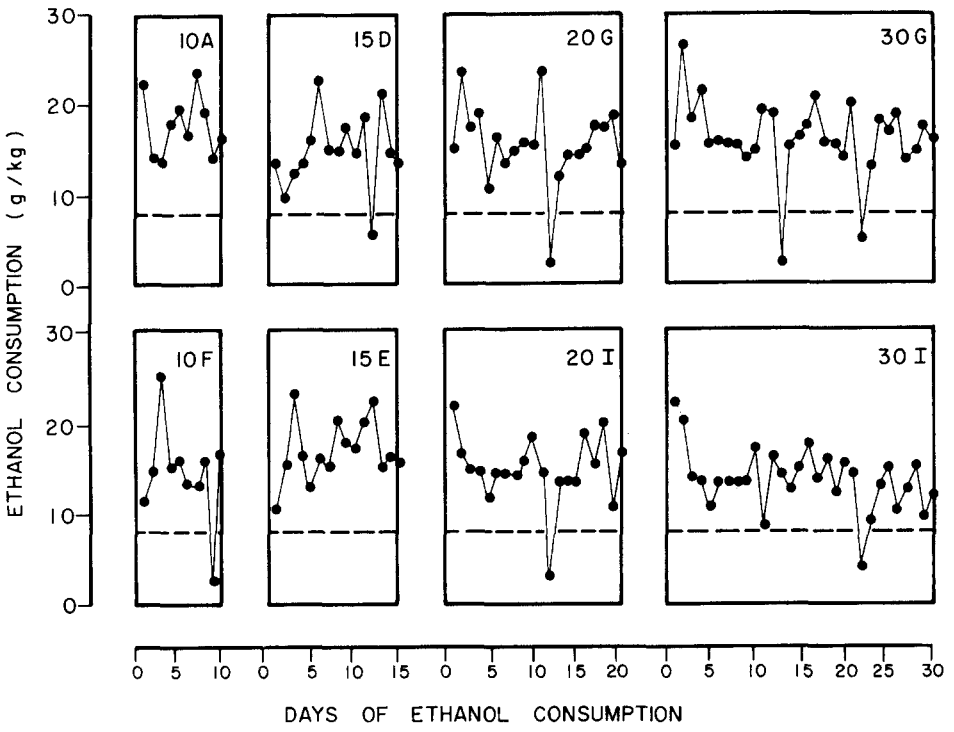

FIG. 2. Individual patterns of ethanol consumption for 2 rats from each group (10, 15, 20, or 30 days consumption). Letters represent individual animal designations within each group. The dotted line represents an ethanol consumption of 8.0 g/kg/day.

Blood Ethanol Concentration

Mean 8:00 a.m. blood alcohol levels (BALs) are shown in Fig. 4. These results indicated a progressive increment in BALs as the duration of ethanol exposure increased. One-way analysis of variance revealed that the increase in BALs over the duration of ethanol exposure was statistically significant ($p<0.001$). Twenty-four hr BALs in the present experiment were partially confounded by spontaneous abstinence periods in 3 rats. These periods were found to be highly correlated with BALs (range: 0.0–78 mg/100 ml). Mean 24–hr BALs determined on the remaining rats were: 8:00 a.m., 264.2 mg/100 ml (range: 235–281); 2:00 p.m., 192.2 mg/100 ml (range: 78–308); 8:00 p.m., 240 mg/100 ml (range: 84–298); and 2:00 a.m., 297.3 (range: 202–379). These results are consistent with previous reports using the liquid diet technique in mice [11], since BALs were elevated throughout the day with a peak during the dark phase of the light cycle. These results are consistent with the hypothesis that physiological dependence is contingent upon continuous exposure of the central nervous system to ethanol [10,35].

Alcohol Withdrawal

The removal of ethanol resulted in the time-dependent appearance of a variety of withdrawal symptoms. The time course and the types of symptoms observed will be discussed within the framework of a behavioral rating scale of withdrawal severity in the rat, developed in our laboratory. The rating scale to be described subsequently arranges a variety of preconvulsive withdrawal signs into symptom complexes (stages) of progressive severity which develop in a coordinated and time-dependent fashion. Convulsions, if they occurred, were divided into 3 stages. The discussion is designed to be as detailed as possible in order to facilitate the ease of recognition of rat withdrawal symptoms among investigators in other laboratories. The rating scale is based on the careful observation of alcohol withdrawal in approximately 125 rats [18, 19, 20, 21], including those in the present experiment, during the past year.

Preconvulsive Stages

Stage I. After the removal of ethanol, the behavior of some rats appeared essentially normal for several hours. Often they continued to exhibit signs of ethanol intoxication in periods ranging from 1–2 hr PW. The initial signs of alcohol withdrawal began 2–5 hr PW. The two most prominent signs of this stage were piloerection and the tails appearing stiff and being carried just above the floor. This sign was especially prominent during movement and may reflect autonomic overactivity, since we have observed similar tail signs in highly aroused rats.

Stage II. The signs most characteristic of this stage were tail arching and a broad-based gait. The severity of tail signs increased markedly: the tail became extended at a 45° angle from horizontal and often was held in an arched position and nearly touched the hindquarters of the animal. Ataxia was reflected by a wide-based, irregular rigid gait, which rendered movement more difficult as this stage progressed. The broad-based gait rendered the appearance of movement as being slow and deliberate. (The broad-based ataxic gait that appeared during this stage was distinct from the ataxia seen during intoxication.) These symptoms

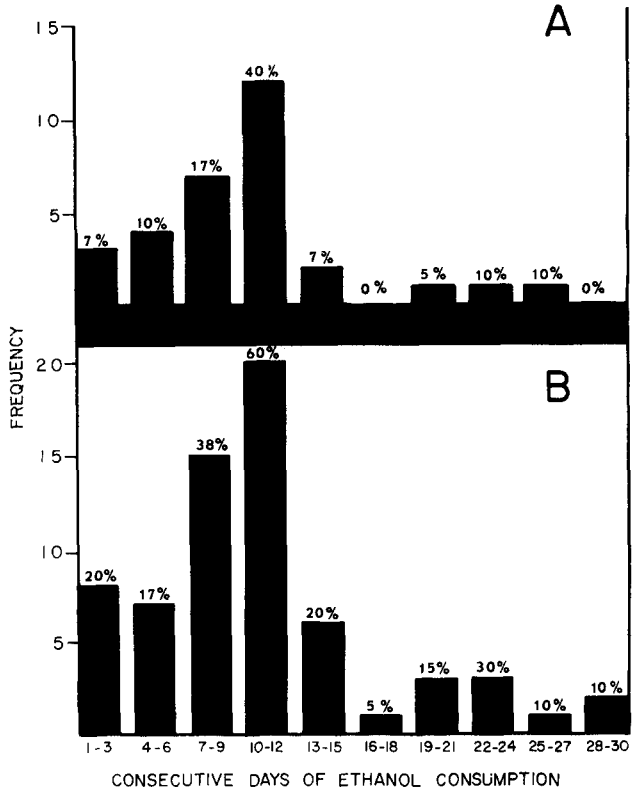

FIG. 3. Frequency distributions showing the periodicity of ethanol consumption. (A) Number of continuous periods of ethanol consumption bounded by a spontaneous withdrawal episode. A spontaneous withdrawal episode was defined as any day in which consumption fell below 8.0 g/kg. (B) Number of continuous periods of ethanol consumption bounded by spontaneous withdrawal episodes or by scheduled withdrawal. The percentage at the top of each vertical bar indicates the proportion of rats displaying spontaneous abstinence periods out of the total number of rats exposed for the given period of time. The percentages do not total 100% because one rat could have more than one spontaneous abstinence episode.

normally began 3–6 hr PW. Some aspects of normal behavior continued during this stage. Some rats continued to groom normally and often exhibited appetitive behavior. However, sleep, while a common feature of Stage I behavior, became fragmented during this stage, occurring only in short bursts interspersed with periods of activity. This sleep fragmentation has been verified electrographically [20] as well as behaviorally.

Stage III. Signs of alcohol withdrawal increased markedly, beginning 5–8 hr PW. This stage was also characterized by insomnia, anorexia, adipsia, infrequent periods of grooming, and a conspicuous absence or diminution of normal behavior. While previous stages were characterized primarily by symptoms indicative of alterations in autonomic activity, a major feature of this stage was the onset of a variety of signs indicating motor dysfunction, including hypoactivity, extensor rigidity, and fasciculations of axial musculature. In the most severe cases, extensor rigidity resulted in the complete extension of the limbs with the abdomen flush against the floor of the observation chamber. Muscular fasciculations often resembled mild tremors but consisted of bursts of rhythmic jerking of the back

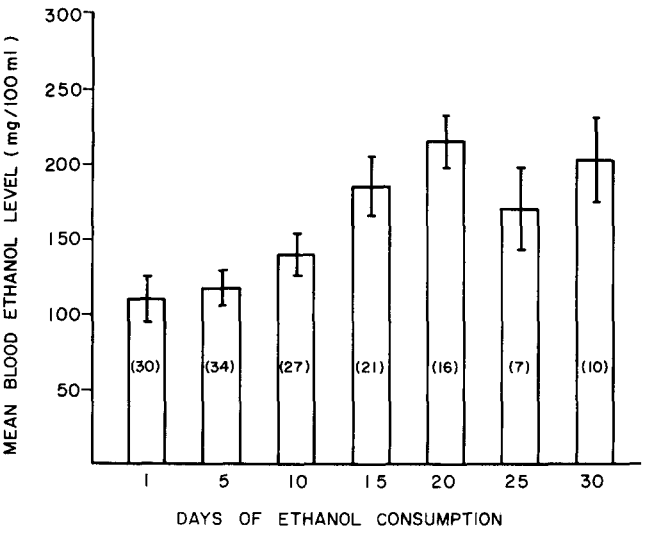

FIG. 4. Mean blood ethanol concentration as a function of days of ethanol consumption. Brackets delineate the standard error of the mean. Numbers in parentheses indicate the number of rats for each point. Blood samples were taken at 8:00 a.m.

muscles. A marked decline in all behavioral activity was a prominent sign. For extended periods of time, the rats often assumed unusual postures against the side of the observation chamber and they began to exhibit increases in hyperreactivity to various visual and auditory stimuli during this stage. A gentle tap on the side of the observation chamber was sufficient to elicit an exaggerated startle response and vocalizations.

Stage IV. The signs exhibited during this stage were among the most unusual and dramatic observed in the rats. Sudden sprawling movements were the primary symptom which began with a series of rapid steps followed by complete tonic forelimb and hindlimb extension, opisthotonus, vocalizations, followed by severe tremors. These movements normally occurred spontaneously but could be elicited by routine laboratory sounds. Motor activity consisted almost entirely of the sprawling episodes. These episodes are probably identical to the "frantic rushing and jumping" movements described by Wallgren [39] after 18–21 days of ethanol intubation in rats; also, similar sprawling episodes have been observed in mice [10]. Stage IV signs were not observed in all animals, but in the rats that did exhibit Stage IV signs, the time course of development of withdrawal symptoms was altered. In these rats Stages I–III were often collapsed in time, with Stage IV signs beginning as early as 4–6 hr PW. This alteration in the time course of the development of withdrawal symptoms could not be attributed to differences in the blood ethanol concentration at withdrawal. Furthermore, Stage IV was characterized by a rapid decline in hyperexcitability, as measured by susceptibility to audiogenic convulsions. Spontaneous convulsions, when observed, usually occurred subsequent to sprawling episodes, and audiogenic convulsions were often elicited during the early portions of Stage IV. Thus, the early portions of Stage IV were usually characterized by a high level of hyperexcitability. However, when Stage IV symptoms often continued for several hours, hyperexcitability declined rapidly. This dissociation between behav-

ioral symptoms and level of hyperexcitability was often dramatic because sprawling movements and severe tremors were evident, yet auditory stimuli failed to elicit a convulsion.

Note that some of the symptoms described in the present report are most clearly recognized during periods of movement. Careful and frequent observations are thus required for the reliable evaluation of preconvulsive signs of withdrawal intensity.

Convulsive Stages

Three convulsive stages were differentiated: *Stage V,* audiogenic convulsion; *Stage VI,* spontaneous convulsion; and *Stage VII,* convulsion ending in death.

The intensity of hyperexcitability developed during alcohol withdrawal was not sufficient for the reliable manifestation of spontaneous convulsions. We observed 2 spontaneous convulsions in the present experiment and have observed only 4 in approximately 125 rats undergoing alcohol withdrawal within the past year. Furthermore, the spontaneous convulsions observed were characteristically mild, with durations of no more than 10–25 sec. Spontaneous convulsions, when they occur, often develop after a sprawling episode and begin with automatisms, especially involving the head and vibrissae, culminating in loss of righting and in whole-body tonic-clonic limb movements.

Audiogenic convulsions were induced during Stage III-IV preconvulsive withdrawal severity. They were preceded by running episodes, ranging from 10–30 sec in duration. The behavioral components of audiogenic convulsions were of at least four types: (1) whole-body tonus; (2) whole-body clonus; (3) forelimb-tonus and hindlimb-clonus; and, (4) forelimb-clonus and hindlimb-tonus. Within a discrete convulsive sequence two or more of these patterns were often exhibited. The pattern of convulsion may be important. We have observed, in association with each type of convulsion, a slightly different pattern of electrographic seizure activity recorded from cortical and subcortical brain regions [20]. Apparently, the behavioral pattern of convulsions, in addition to such parameters as duration, may provide an indication of the intensity of neural hyperexcitability during alcohol withdrawal.

The initial convulsion following the running episode usually lasted from 45–60 sec and was often followed by an unusual automatism – a series of hopping movements with ears laid back, rapid clonic head movements, and vocalizations. The hopping appeared to result from rapid, intermittent clonic movements of the limbs, which propelled the rat around the observation chamber. While gross observations suggested a strong resemblance between this response and species-typical goal-directed behavior, the rats were unresponsive to visual and auditory stimuli during these periods. These results suggest that the hopping movements represent an involuntary postconvulsive behavior, a hypothesis consistent with electrographic recordings during this behavior [20]. A similar postconvulsive automatism consisting of exaggerated motor movements, spasticity, etc., has been reported by Falk, *et al.* [7,8].

In several rats additional discrete periods of behavioral convulsions, masticatory seizures, or unilateral clonic limb movements occurred in periods interspersed between episodes of hopping automatisms. For example, we have observed as many as six discrete convulsions after presentation of a single audiogenic stimulus, the final convulsion ending in respiratory arrest and death. The stages of withdrawal severity and associated symptoms are summarized in Table 1.

Table 2 shows the maximum stages of withdrawal intensity displayed by rats in the 10D, 15D, 20D, and 30D groups. As few as 10 days of ethanol exposure was sufficient for the development of severe withdrawal signs. As shown in Table 2, 50% of the rats from the 10D group were judged to have Stages III–IV withdrawal signs. Further increases in ethanol exposure did not markedly alter the intensity of preconvulsive withdrawal symptoms (Stages I–IV) but increased the percentage of rats exhibiting the most severe abstinence signs (Stages III–IV). The major feature that distinguished rats receiving varying durations of ethanol exposure was observed in the convulsive stages. An increased incidence of convulsions, as a function of duration of ethanol exposure, reached asymptote at approximately 20 days (Table 2). A χ^2 test indicated a significant difference in the number of convulsions among the 4 groups ($p<0.05$). Based on these results, 10 days of ethanol exposure are probably sufficient for the development of ethanol dependence, as indicated by the presence of behavioral abnormalities following ethanol withdrawal. Further increases in ethanol exposure did not appear to result in concomitant increments in the intensity of these behavioral signs but, instead, resulted in a marked growth in hyperexcitability, evidenced by susceptibility to audiogenic convulsions.

We further examined withdrawal severity among the 4 groups in relation to several other parameters assessed during the present experiment. Table 3 compares values for each group obtained for withdrawal intensity, ethanol consumption, BAL recorded at alcohol withdrawal, and the rate of ethanol elimination.

Mean ethanol consumption did not vary markedly as a function of duration of ethanol exposure. Furthermore, differences in withdrawal severity could not be attributed to variations in total ethanol consumption. A Spearman rank correlation between ethanol consumption and withdrawal intensity was not significant. The spontaneous abstinence periods described previously were a corollary feature of ethanol consumption that may have influenced the manifestation of physiological dependence. Rats that underwent such an episode 1 or 2 days before a scheduled withdrawal period showed a diminished withdrawal intensity. However, a Spearman rank correlation between the number of days of consecutive ethanol consumption (without an abstinence period) and withdrawal intensity was not significant. These results indicate that the total duration of ethanol exposure is a more critical determinant of the intensity of withdrawal abnormalities. Partial withdrawal episodes interspersed within a given ethanol exposure period do not appear to influence the ultimate level of behavioral hyperexcitability unless the intensity of physiological dependence is assessed 24–48 hr after a spontaneous abstinence period.

Mean BALs at the time of alcohol withdrawal are shown in Table 3. A Spearman rank correlation between initial BALs and subsequent withdrawal intensity was significant ($r = 0.38$, $z = 2.27$, $p<0.012$). The significance of this correlation between 8:00 a.m. BALs and withdrawal severity is obscured by the fact that BALs also increased as a function of ethanol exposure duration. However, assuming that the 8:00 a.m. BALs provide, in part, an indication of blood ethanol concentration maintained over a 24-hr period, then those rats maintaining higher 8:00 a.m. BALs could subsequently develop the most severe withdrawal symptoms.

TABLE 1
CRITERIA FOR BEHAVIORAL STAGE OF WITHDRAWAL

Stage	Symptoms	Persisting Symptoms	Onset (Hr PW)
Preconvulsive			
I	Tail stiffening Piloerection	–	2–5
II	Tail arching Broad-based gait	Piloerection	3–6
III	Hypoactivity Extensor rigidity Muscular fasciculations Hyperreactivity	Piloerection Tail arching Broad-based gait	5–8
IV	Sprawling episodes Spontaneous vocalizations Whole-body rigidity Severe tremors	Piloerection Tail arching Broad-based gait Extensor rigidity Hyperreactivity	4–8
Convulsive			
V	Audiogenic convulsion	–	–
VI	Spontaneous convulsion	–	–
VII	Convulsion ending in death	–	–

TABLE 2

NUMBER OF RATS IN EACH GROUP DISPLAYING EACH BEHAVIORAL STAGE OF WITHDRAWAL

	Maximum Stage of Withdrawal								
Group	0	I	II	III	IV	V	VI	VII	No. of Convulsions
10 day	0	1	3	3	2	1	0	0	1
15 day	0	1	2	4	0	3	0	0	3
20 day	0	0	1	1	1	3	2	2	7
30 day	0	1	3	1	0	4	0	1	5

Previous reports that have examined the relationship between BALs and ethanol dependence have focused on the onset of withdrawal symptoms. In general, these studies have reported the appearance of withdrawal symptoms before the total elimination of ethanol [31]. Figure 5 shows the relationship between decline in BALs and onset of withdrawal signs in a rat from the 20D group. This particular animal was also chosen to illustrate the compression of Stages I–III in an animal developing Stage IV severity. Although withdrawal symptoms usually appeared while substantial ethanol remained in blood, the most severe symptoms (Stages III–VII) usually occurred after total blood ethanol elimination. Figure 5 illustrates that a rapid decline in BAL is sufficient to trigger the onset of withdrawal symptoms. The initial onset of signs of ethanol dependence may also depend upon whether BALs are increasing or decreasing before ethanol is removed. Usually the magnitude of decrease in BALs sufficient for withdrawal symptoms to appear vary between 100–150 mg/100 ml.

Although the induction of metabolic tolerance has been assumed to account only partially for behavioral tolerance after prolonged ethanol exposure [23], no role has been specified for physiological dependence. Since the appearance of withdrawal symptoms was contingent upon a decline in BALs, we reasoned that the nervous system might be sensitive to the rate of decline of blood ethanol. In each rat, the rate of ethanol elimination was determined from the descending limb of the ethanol disappearance curve. Table 3 shows the mean rate of ethanol elimination

TABLE 3

AVERAGE MAXIMUM WITHDRAWAL SEVERITY, ETHANOL CONSUMPTION, BLOOD ETHANOL CONCENTRATION AND ETHANOL ELIMINATION FOR EACH GROUP

Group	Median Withdrawal Stage	Mean* Ethanol Consumption	Mean† Blood Ethanol Concentration at Withdrawal	Mean Rate‡ of Ethanol Elimination
10 day	III	15.0 (0.8)	132.9 (37.0)	61.5 (5.8)§
15 day	III	15.0 (0.6)	156.1 (26.8)	58.7 (5.4)
20 day	V	15.6 (0.5)	223.5 (23.6)	67.0 (3.9)
30 day	IV	13.6 (0.5)	203.3 (28.8)	61.0 (2.8)

*g/kg/day during the 5 days prior to withdrawal.
†mg/100 ml.
‡mg/100 ml/hr.
§Numbers in parentheses are S.E.M.

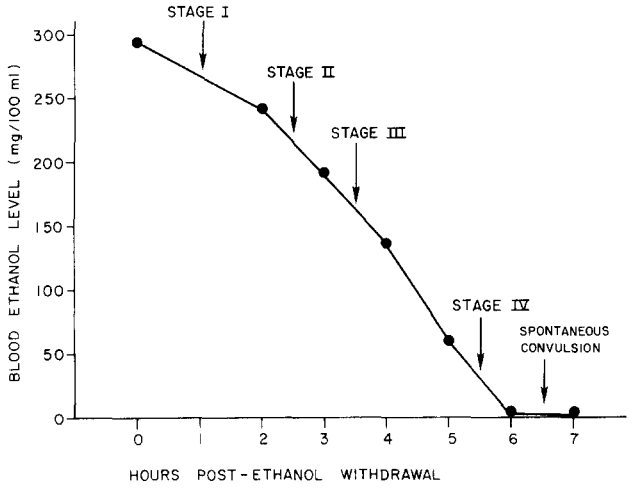

FIG. 5. Decline in blood ethanol concentration and appearance of withdrawal signs as a function of number of hours post-ethanol withdrawal in a rat showing compression of Stages I–III. See text for description of withdrawal stages.

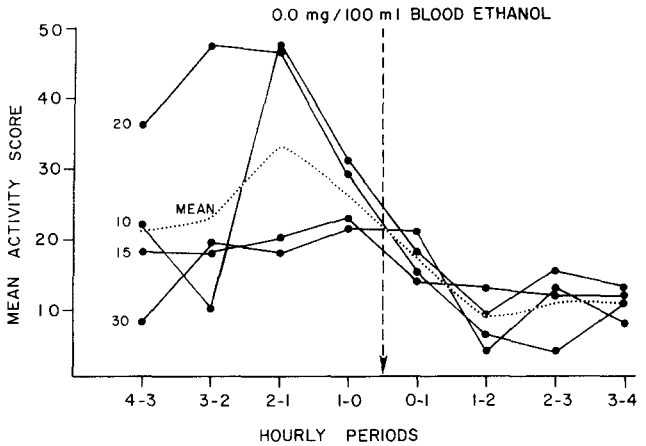

FIG. 6. Behavioral activity during hourly periods preceding and subsequent to the time when blood ethanol reached 0.0 mg/100 ml. The dotted line is the mean of the group scores.

for rats in each of the 4 groups. The hypothesis generated above would predict a direct relationship between ethanol elimination and withdrawal intensity. However, a Spearman rank correlation between the rate of ethanol elimination and withdrawal intensity was not statistically significant. The values obtained for ethanol elimination in the present experiment were substantially higher than those previously reported in the rat [40]. Recent preliminary results [21] indicate that the ethanol elimination rate in the present experiment is increased 50–100% more than control levels. Metabolic tolerance appears to develop gradually and is nearly complete after 10 days of ethanol exposure. Lieber [25] has recently reported a 40% increase of the ethanol elimination rate in rats by using a similar liquid diet procedure.

Behavioral Activity

Activity scores were variable when the time that ethanol was removed was used as the reference point. Since this point is somewhat arbitrary physiologically, activity scores were further analyzed using as a reference the point at which blood ethanol decreased to zero (less than 25 mg/100 ml). The results of this analysis are shown in Fig. 6. A slight peak in activity occurred 1–2 hr before ethanol was totally eliminated. This peak was followed by a progressive decline in activity that was closely associated with total ethanol elimination. These results confirmed impressions based on gross observation, since hypoactivity was included as a Stage III withdrawal symptom. It is unlikely that the variations in activity can be attributed to normal rhythmic variations in activity since total blood ethanol elimination occurred at different points during the observation period across rats. However, alcohol withdrawal *per se*

possibly interacts with normal fluctuations in the resting activity cycle in producing the alterations in activity observed in this experiment.

Duration of Withdrawal Symptoms

We did not systematically study alcohol withdrawal symptoms in rats beyond 12 hr PW in the present experiment. Susceptibility to audiogenic convulsions appears to persist for at least 12 hr PW, declining to near control levels by 24 hr PW (unpublished observations). However, in rats that developed Stage IV signs, susceptibility to audiogenic convulsions decreased much more rapidly. It is not clear what factors determine the appearance and maintenance of Stage IV symptoms or the decline in hyperexcitability.

Several withdrawal symptoms persist for at least 24–48 hr PW in some animals. These include tail signs, mild tremors, hyperreactivity, hyperirritability, and whole-body muscular rigidity. Muscular rigidity was apparent when we handled the animal. We are currently conducting examinations of behavioral and electrographic abnormalities for more extended time periods after ethanol removal.

GENERAL DISCUSSION

The results of this experiment confirm and extend our previous report of ethanol dependence in rats, using the liquid diet technique [18]. Rats, consuming liquid diets containing ethanol for 10–30 days, maintained substantial ethanol intake, leading to behavioral intoxication concomitant with relatively stable blood ethanol concentrations. The removal of ethanol resulted in evidence of physiological dependence, including behavioral manifestations of autonomic and somatic dysfunction and spontaneous and audiogenic convulsions as well. The withdrawal symptoms developed in a characteristic order related to the decline in blood ethanol concentration.

A prominent feature in the results of this experiment was the occurrence of spontaneous abstinence periods. Spontaneous withdrawal episodes ending in convulsion and death have been observed during long-term experiments using the liquid diet technique in mice [12,13]. A more systematic evaluation of this phenomenon within the present experiment revealed an increased probability of spontaneous abstinence periods as a function of duration of ethanol exposure with a tendency toward discrete periods of ethanol consumption with a duration of 7–12 days. By evaluating this phenomenon systematically, we found that spontaneous abstinence periods increased in frequency as the duration of ethanol exposure increased. More specifically, spontaneous abstinence was observed most frequently between 7 and 12 days of continuous ethanol consumption (> 8 g/kg/day). A similar phenomenon has been observed in human alcoholics under experimental laboratory conditions [29]. Alcoholics allowed free access to ethanol show a tendency to separate their drinking periods into discrete episodes, separated by periods of self-imposed abstinence often involving several days [29]. Although it is not clear whether this finding is generalizable outside of the experimental laboratory, this observation has been supported by animal studies. Deneau, *et al.* [3] and, later, Winger and Woods [43] have clearly demonstrated a comparable cyclicity of ethanol consumption in monkeys trained to self-administer ethanol via intravenous infusion. In both situations, abstinence periods occurred despite the presence of withdrawal symptoms. The spontaneous withdrawal episodes observed in rats during ethanol consumption appear to be comparable to the phenomenon observed in man [29] and in monkeys [3,43].

The behavioral rating scale for ethanol withdrawal intensity in rats, described within the present report, is similar to that used previously in mice [10,16]. This scale has proved useful in evaluating absolute withdrawal intensity and in examining temporal characteristics appearing in withdrawal symptoms. Withdrawal signs normally appeared in complexes of overt symptoms, which form the basis for a portion of the rating scale. These symptom complexes developed in a consistent, time-dependent order after ethanol was removed. Perhaps the most reliable behavioral indices of ethanol dependence are tail signs and broad-based gait. These symptoms usually developed soon after ethanol removal and persisted throughout the observation period. A behavioral rating scale of ethanol withdrawal intensity, such as the one reported here, should aid in investigating the underlying mechanisms of physiological dependence on ethanol. For example, a rating scale allows determination of the time course and intensity of the withdrawal reaction. These parameters can then be related to the time course and intensity of alteration in the neurochemical or neurophysiological variables of interest.

Many investigators have used the incidence of convulsions or alterations in startle threshold in assessing ethanol dependence. These experiments have generally shown a decrease in the threshold for startle response [14] or in convulsions induced by electroconvulsive shock [27] or auditory stimuli [18,27]. The symptoms observed during alcohol withdrawal have been interpreted to reflect the release of a latent state of neural hyperexcitability developed from prolonged ethanol exposure [23,31], presumably as a function of one of a variety of proposed cellular adaptive mechanisms [2, 22, 26]. However, using these methods in evaluating withdrawal intensity assumes a unitary neural process in which convulsions represent the maximum in withdrawal severity. Equally conceivable, the various withdrawal symptoms (automatic, somatic, convulsive) may be related to abnormal activity in different brain regions, perhaps occurring as a function of differing cellular adaptive mechanisms. Such a multiregional view of processes underlying the withdrawal syndrome would predict that convulsive susceptibility or motor reactivity alone, used as measures of withdrawal intensity, might result in an incomplete evaluation of the severity of alcohol withdrawal. Thus, in experiments involved with the ethanol withdrawal syndrome, it is desirable to use as many measures of withdrawal severity as possible until more is understood about the neural processes underlying ethanol dependence.

A direct relationship was found between the duration of ethanol exposure and subsequent alcohol withdrawal severity, supporting similar observations made in other species [5,15]. Ten days of ethanol exposure was sufficient for the development of signs of ethanol dependence during the withdrawal period. Further increases in ethanol exposure resulted in increments in the percentage of animals exhibiting severe preconvulsive withdrawal symptoms (when those rats that subsequently had convulsions are included) and a markedly enhanced susceptibility to audiogenic convulsions as well. Withdrawal intensity appeared to asymptote at 20 days, since further increases in severity were not apparent after 30 days of ethanol exposure. It should be emphasized that the results of the present experiment do

not provide a time constant for the development of ethanol dependence in the rat. Dose-response curves for the development of ethanol dependence have previously been demonstrated to depend upon the dosage of ethanol as well as the duration of exposure [15]. Thus, using other techniques that provide for a greater or smaller effective dosage should result in a different time course in the development of ethanol dependence.

A direct relationship was observed between BALs at withdrawal and subsequent withdrawal intensity. However, the BAL also increased as a function of duration of ethanol exposure, thus complicating interpretation. Curiously, 8:00 a.m. BALs continued to increase as a function of duration of exposure without concomitant total increases in ethanol consumption, despite increases of approximately 50–100% in ethanol elimination rate. This increased BAL could be a function of a buildup of residual nonmetabolized ethanol. Even when an ethanol elimination rate twice that previously reported in the rat [40] is considered, the rats consistently consumed quantities of ethanol above that which could be metabolized throughout the ethanol exposure period. It is conceivable that the diurnal pattern of ethanol intake changes as a function of duration of ethanol exposure. Nevertheless, a sufficiently elevated BAL, together with the level of cellular adaptation induced as a function of duration of ethanol exposure, may interact in the subsequent manifestation of physiological dependence.

The liquid diet technique, as originally described in mice [10], was criticized for the severe weight reduction, and the nutritional adequacy of the liquid diets was questioned [30,33]. These criticisms do not apply to the present experiment. The weight reduction reported here is comparable to that used in inducing ethanol dependence with other techniques [7, 8, 33]. The rats gained weight throughout the liquid diet treatment period and showed no evidence of malnutrition. Furthermore, the rats had reattained predeprivation weight after approximately 20 days of diet consumption. The nutritional adequacy of the liquid diets used in the present experiment has been documented in detail previously [36]. The liquid diets contain more than the recommended daily requirements for proteins, vitamins, and minerals in the growing rat [41]. Furthermore, the diets were fortified with additional vitamins and minerals. We have previously calculated [36] that rats consuming these liquid diets obtain 3–34 times the minimum daily requirement of vitamins and nutrients [41]. In addition, we have consistently observed in long-term experiments (4–9 months) that rats consuming ethanol or sucrose liquid diets gain more weight than rats fed pelleted laboratory food and water ad libitum [36, 37, 38].

The original study by Ogata, *et al.* [33], which has served as the basis for much of the criticism of the liquid diet technique developed for mice [10], compared mice consuming ethanol in liquid diets with mice consuming aqueous ethanol solutions via a polydipsia technique. Despite the consumption of large quantities of ethanol, the polydipsia group failed to develop signs of ethanol dependence that were in marked contrast to withdrawal symptoms, including convulsions observed in the liquid diet group. These differences could not be attributed to variations in liver histopathology or in strain, age, duration of ethanol exposure, or blood ethanol concentrations. At least two hypotheses were forwarded to account for the discrepancy: (1) nutritional deficiency in combination with severe weight loss or (2) variations in the diurnal pattern of ethanol intake. The recent successful demonstration of ethanol dependence using a polydipsia technique [7,8], which resulted in stable 24-hr blood ethanol levels, suggests that the latter hypothesis could more likely account for the differences observed by Ogata, *et al.* [33]. Earlier experiments with rats had also failed to demonstrate physical dependence by using the polydipsia technique [17, 24, 28]. It appears safe to conclude that the signs of ethanol dependence observed in the present experiment can be attributed to the central nervous system effects of ethanol and not to the initial weight reduction or nutritional deficiency.

The potential significance of formulating a model for study of ethanol dependence in the rat has led to considerable experimental effort. Recent successful reports of ethanol dependence in rats have involved techniques in which ethanol is administered by inhalation (Littleton and Griffiths personal communication), intubation [39], liquid diets [1, 18, 25], or using a modified schedule-induced polydipsia technique [7,8]. Using varied techniques for administering ethanol is useful in that it provides an experimental control for any possible confounding variables inherent in any single model. Note that many of the withdrawal symptoms described within the present experiment appear to be similar to those reported using the intubation [39] and polydipsia [8] techniques. Each of the above models appears to have its advantages and disadvantages, with the ultimate choice of a model contingent upon the experimental question under investigation.

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