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BRIEF COMMUNICATION

Pattern of Daily Blood Ethanol Elevation and the Development of Physical Dependence[1]

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SAMSON, H. H. AND J. L. FALK. *Pattern of daily blood ethanol elevation and the development of physical dependence.* PHARMAC. BIOCHEM. BEHAV. 3(6) 1119–1123, 1975. – Schedule-induced ethanol polydipsia regimens were used which produced either one or two daily peaks in blood ethanol levels. After 3 months on these regimens, rats were withdrawn from ethanol and tested for the presence of abstinence signs. No evidence of physical dependence was found, a result which contrasted with the previous finding of a severe withdrawal syndrome when blood ethanol was maintained at a more continuously elevated level prior to withdrawal. It was concluded that, as in the case of barbiturates, the development of physical dependence on ethanol requires more than an episodic peaking of the blood ethanol level once or twice per day.

Ethanol intake Ethanol physical dependence Abstinence Blood ethanol Schedule-induced polydipsia Adjunctive behavior

WHEN high blood ethanol levels were maintained by schedule-induced ethanol polydipsia in rats for 3 months, ethanol withdrawal revealed a severe state of physical dependence resulting in deaths from tonic-clonic convulsions [5,6]. In contrast to this procedure, which maintained high blood ethanol levels for most of each 24 hr cycle, high daily ethanol intakes yielding a single daily peak failed to produce dependence [14]. It has been suggested that for the development of physical dependence on certain non-opiate drugs continuous maintenance of high blood levels must occur (for a review see [12]). While the evidence that this is the case for the barbiturates is strong [17], the situation with respect to ethanol remains unclear.

In their classic study on the development of ethanol dependence, Isbell *et al.* [11] found that after 48 days of chronic ethanol intake all subjects had moderate to severe withdrawal symptoms. Daily ethanol was administered to maintain a moderate to high blood level, and withdrawal severity correlated well with the daily amount of ethanol ingested. The authors concluded that the maintenance of a high blood level was necessary to prevent the onset of withdrawal symptoms. However, they did not examine whether this continuous maintenance was a requirement for the production of the state of dependence itself.

Animal studies producing unequivocal physical dependence have all used methods which resulted in the maintenance of high blood ethanol throughout each day. Thus, the stomach loading [3,4], intravenous self-administration [2,16], inhalation [10], liquid diet [8], and schedule-induced polydipsia [5,6] methods have all led to rather continuously elevated blood ethanol levels, either through programmed loading or self-administration. While it is reasonable to assume that other studies in which dependence was not demonstrated failed owing to the occasional or episodic nature of the blood ethanol elevations, the case has never been proven. Most such studies typically have involved rather low daily ethanol intakes as well, so that pattern and amount of intake have been confounded.

The present experiment utilized schedule-induced ethanol polydipsia arrangements resulting in high, but episodic, intakes over each 24 hr cycle to determine if such periodic peaking, rather than continuous maintenance, of the blood ethanol level could produce physical dependence.

METHOD

Animals

Fourteen adult male, albino rats (Holtzman strain) with a mean starting body weight of 355.5 g ± 4.5 g were used. Over a two-week period, they were gradually reduced to 80

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percent of their free-feeding body weight by restricting daily food intake.

Experimental Environment

The animals were housed in Plexiglas chambers similar to those used in previous studies [5, 6, 14]. The chambers contained a food cup that was connected to a Gerbrands pellet dispenser which could deliver 45-mg food pellets (P. J. Noyes Co., Lancaster, N. H., Lab rat food diet, 4.3 kcal/g) automatically. A single, stainless-steel drinking spout (Ancare, TD-300) with a double ball bearing arrangement to prevent fluid leakage was attached to a 250 ml graduated cylinder which contained the only available fluid in the chamber. There was 24 hr fluorescent lighting. The animals remained in these chambers 24 hr per day for the duration of the experiment.

Procedure

After reduction to the 80 percent body weight level, the animals were placed into the experimental chambers. They were divided equally into two groups and were placed on one of two feeding regimens. Group one (GI) received two, 1 hr pellet delivery periods per day. Food pellets were delivered every 2 min during these 1 hr intervals (a fixed time 2 min schedule). The two food pellet delivery periods started at 9:00 a.m. and 9:00 p.m. daily. Group two (GII) received the identical two food pellet delivery periods, but at 5:00 a.m. and 9:00 a.m. Thus, both groups received 60 food pellets per day in two, 1 hr delivery sessions. The only difference between the groups was the time between the 2 delivery sessions: GI had a 12 hr separation, while GII had a 3 hr separation.

At 10:30 a.m. daily, the animals were weighed, their fluid intakes recorded and fluid reservoirs refilled. An additional 120 Noyes 45-mg food pellets were placed into the food cup, which equated the total daily food ration with the amount used in previous studies [5,14]. The only fluid available was 5 percent ethanol (v/v). During the last 72 hr of the experiment, water was substituted for the ethanol, so that the effects of ethanol withdrawal could be assessed.

During the second month, blood samples were obtained at various times before and after each feeding (see Figs. 1 and 2) to determine blood ethanol levels. Samples were taken at 8:00 a.m. to 6:00 p.m. for all animals on one day, while 6:00 p.m. to 8:00 a.m. samples were taken 7 days later. Blood ethanol levels were determined by the enzymatic method [1].

After 3 months of ethanol ingestion on the above feeding regimens, the animals were withdrawn from ethanol by substituting water as the only available drinking fluid. Continuous observation of the animals was maintained over the first 15 hr of withdrawal. Between 7–10 hr of ethanol withdrawal, all animals were tested for seizure susceptibility by a shaking of keys (as previously described [5,6]). Their behavior was monitored periodically for the next 72 hr, in order to assess any withdrawal seizures which might occur.

TABLE 1

BODY WEIGHT (g), 5 PERCENT ETHANOL INTAKE (ml), AND g ETHANOL INTAKE/kg BODY WEIGHT FOR RATS ON TWO FEEDING REGIMENS FOR EACH OF 3 MONTHS

	Group I (2 sessions, 9 a.m. & 9 p.m.) N = 7	Group II (2 sessions, 5 a.m. & 9 a.m.) N = 7
Month 1		
weight (g)	287 ± 4.3	292 ± 4.8
intake (ml)	62.2 ± 4.2	60.3 ± 6.0
g/kg	8.6 ± 0.5	8.2 ± 0.8
Month 2		
weight (g)	300 ± 5.1	307 ± 4.6
intake (ml)	74.8 ± 3.2	73.2 ± 3.0
g/kg	9.9 ± 0.3	9.5 ± 0.3
Month 3		
weight (g)	312 ± 4.1	322 ± 6.2
intake (ml)	77.0 ± 2.9	77.0 ± 3.2
g/kg	9.8 ± 0.3	9.5 ± 0.3

RESULTS

The mean monthly data are presented in Table 1. All animals gained weight over the 3 month period. They showed an increase in their daily ethanol intake which was proportionally greater than the concomitant weight gain, thereby increasing their g/kg ingested daily. No significant differences were found between the two feeding regimens in terms of intake volume, weight gain, or g ethanol/kg/day during the 3 months of the experiment.

Figure 1 presents the blood ethanol levels for GII. Here, blood ethanol rose after the first pellet delivery session (5–6 a.m.) and remained high throughout the next pellet session at 9–10 a.m. Thus, there was only a single blood ethanol peak every 24 hr.

Figure 2 presents the mean blood ethanol levels for GI. It is clear that prior to each feeding session, the blood levels were low, and that following the pellet delivery session, the blood levels of ethanol were markedly elevated. Thus, with 2 feedings 12 hr apart, there was a cyclic rise and fall in blood ethanol every 12 hr.

Curves fitted by point approximation (Fig. 3A and B) to the points of Figs. 1 and 2 emphasize the two discrete peaks of GI as compared to the single peak of GII. However, computation of the areas under each curve showed only a slight difference between the two groups, indicating that the time spent each day at any given blood ethanol level was approximately the same for both groups. Thus, while two distinct patterns of intake were clear, there was no significant difference in amount of ethanol intake or blood levels attained other than the distribution of those levels during the 24 hr period.

The withdrawal from ethanol after the 3 months of intake failed to show any signs of an abstinence syndrome in either group. There were no signs of increased excitability, and no seizures could be elicited by the shaking of keys which had previously, under another feeding regimen, produced severe and fatal convulsions [5, 6, 7].

DISCUSSION

The daily patterns of blood ethanol elevation were directly related to the pellet delivery regimens. Even with only 2 daily pellet delivery sessions, instead of the 6 used previously [5,6], large quantities of ethanol were consumed and blood ethanol was markedly elevated during the one or two daily peaks. The blood ethanol peaks were related to the pellet delivery sessions clearly showing the control the schedule-induction procedure exerts on ethanol intake. By controlling the delivery pattern of food pellets one can gain a rather close control over blood ethanol levels throughout each 24 hr cycle.

Even though large quantities of ethanol were ingested daily, the lack of withdrawal symptoms suggests that either the intakes were not quite great enough or that the temporal distribution of drinking was such as to not maintain the elevated blood ethanol levels long enough during each 24 hr to produce physical dependence. It is instructive to compare this study with our previously published data [6,14]. Figure 3 (C and D) compares blood ethanol curves generated by a single, large daily food ration (C) [14], or six, 1 hr schedule-induced polydipsia sessions per day (D) [6] with the two conditions in the present study (Fig. 3, A and B). The total areas under these curves show that all of the three conditions not producing physical

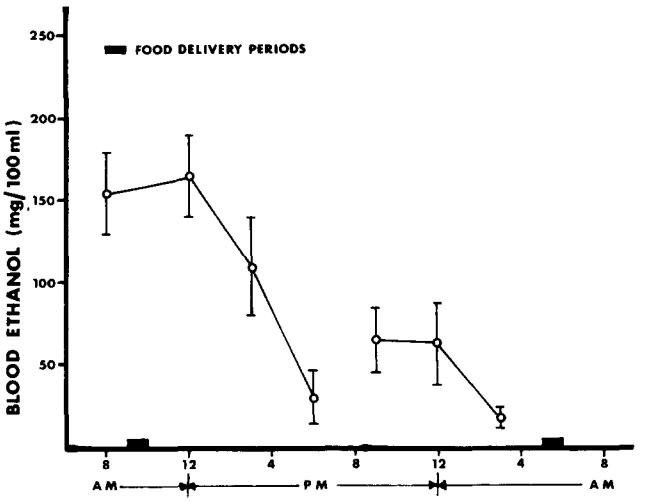

FIG. 1. Blood ethanol concentrations for Group II (N = 7).

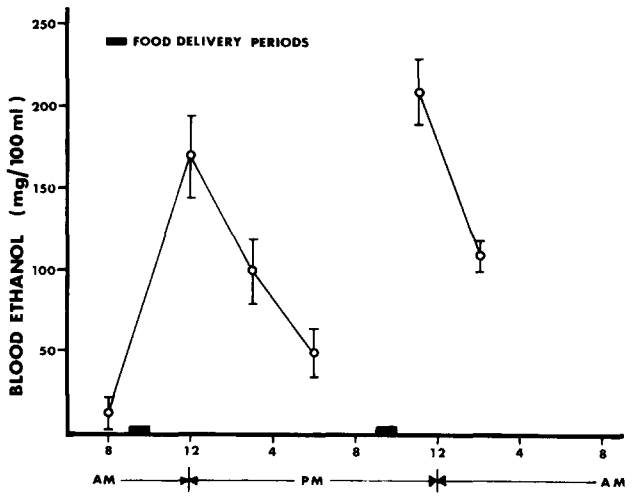

FIG. 2. Blood ethanol concentrations for Group I (N = 7).

dependence (A, B, C) have less than 70 percent of the area of the condition which did produce physical dependence (D of Fig. 3 and Table 2). At the same time, all animals' intakes were 70 percent or greater of the condition producing physical dependence, with the group that had the largest difference in area having the least difference in intake; the single daily ration group (C of Fig. 3).

If the mean blood level for the condition that produces physical dependence (D of Fig. 3) is used as a reference level, all other conditions were well below this level for most of each 24 hr period (Table 2). If the lowest blood level found in the condition producing physical dependence is used as a reference, only one group (GI) reaches this level for over 50 percent of any 24 hr period (Table 2). It does this, however, on the next to smallest ethanol intakes observed. Therefore, it seems suggestive that not just amount, but the distribution of drinking within a 24 hr period plays an important role in the daily blood ethanol pattern. It is not possible to uncouple the amount of ethanol intake from the temporal distribution of this

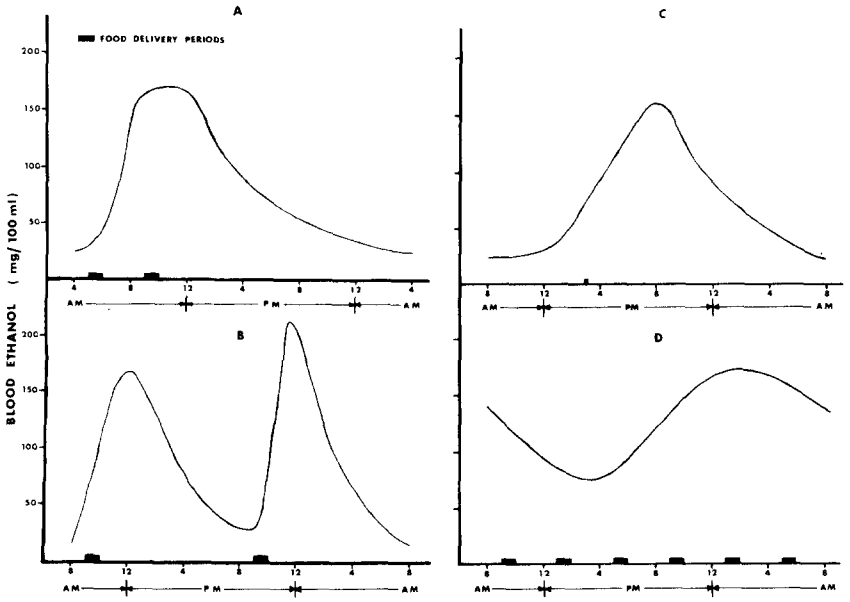

FIG. 3. Temporal distributions of blood ethanol concentrations (point approximation curves) under various daily feeding regimens: (A) group I, (B) group II, (C) single, fixed ration [14], (D) 6 schedule-induced polydipsia sessions [6].

TABLE 2

MEAN ETHANOL INTAKE UNDER 4 DIFFERENT FEEDING REGIMENS AND COMPARISON OF AREAS UNDER 24 HR BLOOD ETHANOL CURVES (BEC)

Group	g/kg/day	Percent g/kg of G4	Percent BEC Area of G4	Percent Time of Blood Ethanol Above Mean of G4 (= 115 mg %)	Percent Time of Blood Ethanol Above Lowest Point of G4 (= 75 mg %)
G1 (9 a.m.–9 p.m., 2 sessions)	9.4	71	68	33	54
G2 (5 a.m.–9 a.m., 2 sessions)	9.1	70	62	29	46
G3 (one daily ration)*	11.7	89	59	25	42
G4 (1,5,9 a.m. & p.m., 6 sessions)†	13.1	100	100	50	100

*Data from [14] †Data from [6]

intake, but it would appear that just a high intake of ethanol, between 9 and 12 g/kg/day (this study, 14), is not in and of itself sufficient to produce physical dependence.

Several investigators [11, 12, 13, 15, 17] have suggested that a maintained daily blood level of certain substances is a necessary condition for the development of physical dependence.

Data from the present studies and others [2, 3, 4, 6, 8, 9, 10, 13, 14, 16] support this hypothesis and indicate that high daily intake of ethanol alone is not sufficient for the production of physical dependence.

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