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The Development of Metabolic Tolerance in the Alcohol-Preferring P Rats: Comparison of Forced and Free-Choice Drinking of Ethanol[1]

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LUMENG, L. AND T.-K. LI. *The development of metabolic tolerance in the alcohol-preferring P rats: Comparison of forced and free-choice drinking of ethanol.* PHARMACOL BIOCHEM BEHAV **25**(5) 1013–1020, 1986.—Experiments were performed to determine whether metabolic tolerance to alcohol develops in the alcohol-preferring P rats during free-choice drinking. In Experiment 1, alcohol elimination rates (AERs) in female Wistar and P rats were measured as a function of age from 26 to 180 days old. AERs calculated as mmol hr^{-1} per kg body weight fell with age, whereas AERs expressed as mmol hr^{-1} per rat increased to reach a constant value after 60 days of age. These data indicate that the chronic effects of ethanol on AER are most easily interpreted if experiments are performed in animals 60 days of age or older and AERs are calculated as mmol hr^{-1} per rat. In Experiments 2 and 3, P female rats were exposed to alcohol for 6–7 weeks either by free-choice drinking or by forced feeding with liquid diets. With free-choice drinking of alcohol, solid food containing 31 percent of the calories as protein, 10 percent ethanol (v/v) and water were made available ad lib. The liquid diets used for forced ethanol feeding were the Bio-Serv-711 diet, a protein-supplemented Bio-Serv-711 diet and the AIN diet and they contained 18, 32 and 22 percent calories as protein, respectively. When compared with pair-fed or ad lib controls, all the P rats exposed to alcohol by either free-choice or forced-feeding exhibited increased AERs (i.e., metabolic tolerance) after 6–7 weeks. However, if AERs before and after alcohol exposure in the same animals were compared, a net increase in AER was evident only in the P rats on free-choice drinking or forced-fed diets which contained at least 22 percent protein. Alcohol consumption and blood alcohol concentrations of the P rats exhibited diurnal variation during free-choice drinking or when they were forced-fed alcohol diets which contained at least 22 percent protein. The high BACs attained in the P rats given the ethanol-containing Bio-Serv-711 diet, presumably because of the lower AERs under this condition, disrupted the diurnal cycling of alcohol ingestion and blood alcohol concentrations. The studies demonstrate that the P rats on chroinic free-choice drinking of alcohol develop metabolic tolerance to much the same degree as animals forced fed ethanol contained in liquid diets. Additionally, they demonstrate that, in animals fed ethanol-containing liquid diets, a net increase in AER after alcohol exposure is evident only if dietary protein constitutes at least 22 percent of the total calories.

Ethanol Metabolic tolerance Alcohol-preferring P rats Alcohol elimination rate Alcohol dehydrogenase

THE P (alcohol-preferring) and NP (alcohol-nonpreferring) lines of rats have been raised by selective breeding as an animal model to investigate the biochemical, physiological and behavioral concomitants of voluntary alcohol consumption [7]. The P rats orally self-administer ethanol solutions (e.g., 10 percent v/v) when food and water are freely available and their blood alcohol concentrations rise to as high as 218 mg percent during the dark cycle [18,30]. Recent studies indicate that blood alcohol concentrations as low as 16 mg percent are pharmacologically active in the P rats since they exhibit a stimulation of spontaneous motor activity after given doses of ethanol that produce peak blood alcohol concentrations in the range of 16–70 mg percent [29]. The P but not the NP rats also self-administer ethanol by the intragastric route in a free-choice situation, when food is available ad lib [27]. Thus, the post-ingestive effects of ethanol is reinforcing. We have also shown that, with chronic free-choice drinking, the P rats develop physical dependence as evidenced by signs of withdrawal when the alcohol solution is removed [28].

The development of tolerance to the effects of ethanol is a well-documented consequence of chronic exposure and its acquisition with free-choice drinking is a necessary criterion for an animal model of alcoholism. We have been exploring

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TABLE 1
COMPOSITION OF DIETS

		Protein	Fats	Carbohydrates	Ethanol	Total
Bio-Serv-711 (BS-711)						
Control	Kcal/ml	0.18	0.35	0.47	—	1.00
	%Kcal	18	35	47	—	—
Ethanol	Kcal/ml	0.18	0.35	0.11	0.36	1.00
	%Kcal	18	35	11	36	—
Supplemented Bio-Serv-711 (SBS-711)						
Control	Kcal/ml	0.39	0.35	0.47	—	1.21
	%Kcal	32	29	39	—	—
Ethanol	Kcal/ml	0.39	0.35	.11	.36	1.21
	%Kcal	32	29	10	29	—
Wayne-Blox solid food (WB)						
	Kcal/g	1.04	0.36	1.99	—	3.39
	%Kcal	31	11	58	—	—
Bio-Serv-AIN-76 (AIN)						
Control	Kcal/ml	0.22	0.115	0.665	—	1.00
	%Kcal	22	11.5	66.5	—	—
Ethanol	Kcal/ml	0.22	0.115	0.31	0.355	1.00
	%Kcal	22	11.5	31	35.5	—

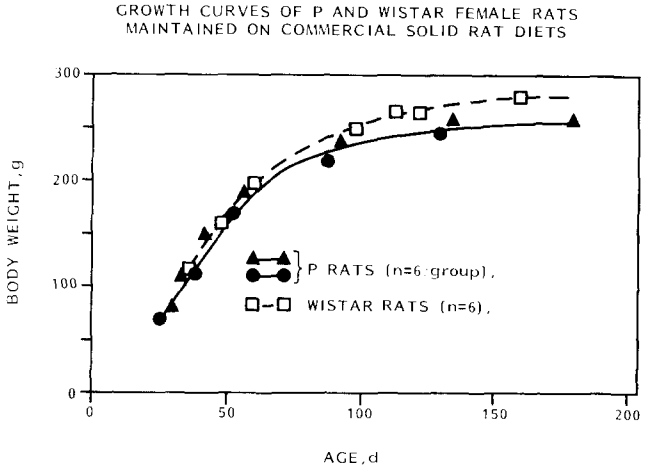

FIG. 1. Growth curves of a group of female Wistar rats and two groups of female P rats maintained on a commercial solid rat diet (Wayne-Blox). The coefficients of variation ranged from 2 to 7 percent.

whether the P rats will develop metabolic and neuronal tolerance to alcohol with chronic free-choice drinking. This report describes studies on the development of metabolic tolerance as a result of chronic ethanol intake. We included as positive controls in these studies, P rats that were forced-fed ethanol incorporated into liquid diets. Because liquid diets differ in protein content [12], we also examined the effect of different diet formulations on the development of metabolic tolerance and on the diurnal variation of alcohol ingestion and blood alcohol concentrations.

METHOD

Animals

Female Wistar and P rats were housed individually in wire-mesh cages and in a controlled temperature and humidity environment with fixed light-dark cycles (7 a.m. to 7 p.m., light and 7 p.m. to 7 a.m., dark). The Wistar rats were purchased from Harlan Industries, Indianapolis, IN and rats from the P line were from the S-21 generation of the alcohol-preferring rats selectively bred in our laboratory. In Experiment 1, the growth curves and alcohol elimination rates (AERs) of 2 groups of P rats and a group of Wistar rats (n=6 per group) were monitored as a function of age (26 day old to 180 day old). The mean age of the P rats at the start of Experiment 2 was 68±2 days and that at the start of Experiment 3 was 60±1 days.

Diets

In Experiment 1, the Wistar and P rats after weaning were given Wayne-Blox food pellets and water ad lib.

In Experiment 2, the P rats were divided into 6 groups (n=6 per group) and given different feeding regimens for 7 weeks. The first two groups were pair-fed the control and the ethanol Bio-Serv-711 (BS-711) liquid diet (Bio Serv, Inc., Frenchtown, NJ). The BS-711 liquid diet was formulated according to the diet composition published by DeCarli and Leiber in 1967 and is similar to the Leiber-DeCarli 1982 regular liquid diet [12]. The third and fourth groups were also pair-fed but the control and ethanol liquid diets were the "supplemented Bio-Serv" diet (SBS-711) described by Rogers *et al.* [22]. The supplementation consisted of adding vitamin-free casein and zinc to the Bio-Serv-711 diet (Table 1). The fifth group was given Wayne-Blox food pellets (WB) and water ad lib, and the sixth group was given ad lib Wayne-Blox food pellet and the free-choice drinking of 10 percent (v/v) ethanol and water. The animals given the liquid

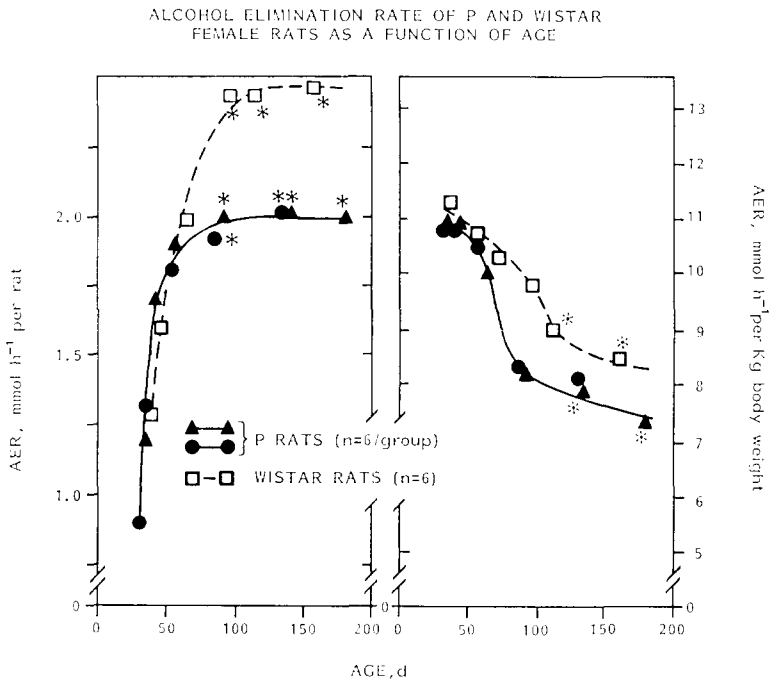

FIG. 2. Change in alcohol elimination rate (AER) of the P and Wistar rats as a function of age. AERs were expressed as either mmol hr $^{-1}$ per rat (left panel) or mmol hr $^{-1}$ per kg body weight (right panel). The coefficients of variation ranged from 1 to 11 percent. Values marked with an asterisk are not different from each other and are statistically different from those without an asterisk.

TABLE 2

MEAN DAILY CALORIC INTAKE IN P RATS GIVEN THE DIFFERENT DIETARY REGIMENS IN EXPERIMENT 2

	Mean daily caloric intake per rat, Kcal/day								
	Week 1			Week 3–4			Week 7		
	Total	Pr	Ethanol	Total	Pr	Ethanol	Total	Pr	Ethanol
BS-711 (C)	50.0*	9.0*	—	51.7*	9.3*	—	58.6	10.6*	—
BS-711 (E)	51.6*	9.3*	18.6	51.7*	9.3*	18.6	59.2	10.6*	21.3
SBS-711 (C)	59.2	19.2	—	59.4	19.2	—	61.3	20.2	—
SBS-711 (E)	60.6	19.6	17.7	59.4	19.2	17.3	61.2	19.8	17.9†
WB (C)	59.3	18.8	—	60.2	19.1	—	58.5	17.0	—
WB (Free-choice)	59.2	14.2	14.4†	60.4	14.2	16.4	59.4	14.0	15.1*

*Significantly different from the SBS-711 (C), SBS-711 (E), WB (C) and WB (Free-choice) diets (by analysis of variance).
†Significantly different from the BS-711 (E) diets (by analysis of variance).
Abbreviations: Pr, protein; C, control; E, ethanol.

diets were acclimated to increasing concentrations of ethanol in the diet in the manner suggested by the manufacturer.

In Experiment 3, the P rats were separated into four groups (n=5 per group). The first two groups were pair-fed the control and the ethanol-AIN liquid diet (Bio-Serv AIN-76 diet) [16,21]. The third group was fed powdered Wayne-Blox solid food and a sucrose solution, while the fourth group was given the powdered Wayne-Blox solid food, water and 10 percent ethanol. The third and fourth groups of rats were pair-fed by providing to the control rats (the third group) the amount of solid food and an amount of the sucrose solution that is isocaloric to the amount of 10 percent ethanol consumed by the rats on free-choice drinking (the fourth group).

Determination of Alchol Elimination Rate (AER) in Vivo

Ethanol (2 g/kg) was administered by the intraperitoneal injection of a 10 percent (v/v) solution. Tail blood samples were collected as a function of time after ethanol injection and the blood ethanol concentrations were measured by a head space method with a gas-liquid chromatograph [13].

TABLE 3

EFFECT OF DIETARY REGIMENS ON ETHANOL INTAKE IN EXPERIMENT 2

Diet	Ethanol Intake, g/kg/day						
	Week 1	2	3	4	5	6	7
BS-711 (E)	10.8 ± 0.5	13.6 ± 1.3	12.5 ± 0.3	12.1 ± 0.4	12.8 ± 0.4	12.9 ± 0.4	13.2 ± 0.2
SBS-711 (E)	11.7 ± 0.4	10.3 ± 0.3*	10.0 ± 0.3*	9.8 ± 0.2*	9.8 ± 0.2*	10.4 ± 0.3*	9.5 ± 0.3*
WB (Free-choice)	8.5 ± 0.6*	8.3 ± 0.3*	9.3 ± 0.7*	7.2 ± 0.7*	7.8 ± 0.4*	11.5 ± 0.7	8.4 ± 0.3*

*Significantly different from the BS-711 (E) group (by analysis of variance).
Abbreviations: E, ethanol.

TABLE 4

EFFECT OF DIFFERENT DIETARY REGIMENS ON BODY WEIGHT AND ALCOHOL ELIMINATION RATE IN EXPERIMENT 2

Diet	Body Weight, g		Alcohol Elimination Rate, mmol/hr/rat		Alcohol Elimination Rate, mmol/hr/kg	
	Before	7th Week	Before	7th Week	Before	7th Week
BS-711 (C)	205 ± 10	204 ± 7	1.90 ± .12	1.66 ± .05	9.25 ± .39	6.95 ± .28
BS-711 (E)	208 ± 3	223 ± 5	2.07 ± .04	2.02 ± .06	9.97 ± .31	9.08 ± .18
SBS-711 (C)	209 ± 7	262 ± 4	1.97 ± .06	1.72 ± .05	9.50 ± .48	6.60 ± .28
SBS-711 (E)	204 ± 6	270 ± 8	1.92 ± .07	2.22 ± .09	9.59 ± .32	8.25 ± .26
WB (C)	208 ± 6	263 ± 4	2.03 ± .05	2.10 ± .13	9.71 ± .28	7.59 ± .50
WB (Free-Choice)	227 ± 12	274 ± 8	2.11 ± .08	2.50 ± .11	9.36 ± .17	9.14 ± .27

Brackets indicate that the compared values are significantly different. Body weight differences were analyzed by analysis of variance. Alcohol elimination rate differences were analyzed by two-tailed t-test.
Abbreviations: C, control: E, ethanol.

The rate of ethanol disappearance was pseudo-zero order, and the AER was calculated using Widmark's equation [6].

Measurement of Liver Alcohol Dehydrogenase (ADH) Activity

Liver homogenate-supernatant fractions were prepared [13] and alcohol dehydrogenase (ADH) activity was assayed spectrophotometrically by measuring the reduction of NAD^+ to NADH. The assay buffer contained 0.5 M Tris-HCl (pH 7.2 and I=0.2), 2.8 mM NAD^+, and 5 mM ethanol, and the temperature was maintained at 37° [14]. These substrate concentrations saturate the enzyme 88 percent; therefore, the measured enzyme activity was multiplied by 1.14 to calculate the maximum ADH velocity (or Vmax) [14].

Statistical Analysis

Results are presented as means±SEM. The two-tailed student's t-test for unpaired data was used for statistical comparisons. Differences were considered to be significant when the p values was less than 0.05. Where results among different experimental groups were compared in Experiment 2, analysis of variance and post-hoc Newman-Keuls tests were used to evaluate statistical differences.

RESULTS

Experiment 1

In previous studies of metabolic tolerance [3, 10, 11, 15, 20, 24, 26], rats were pair-fed control and ethanol-containing diets for several weeks, and the AERs of the two groups were then compared with the results expressed in most studies as mmol of ethanol metabolized per hour per kg body weight. In other studies, only the disappearance rates of ethanol in plasma (mmol/100 ml per hr) were compared. In all of these studies, no attempts were made to discern if ethanol ingestion had actually increased the AER when compared with that before ethanol exposure in the same animals.

To set the stage for Experiments 2 and 3, we determined in Experiment 1 the growth curve of P female rats and the change in AER as a function of age. The purpose was to find the most appropriate reference unit to express AERs for comparison of data. Since the P line of rats was originally

TABLE 5

EFFECT OF DIFFERENT DIETARY REGIMENS ON HEPATIC ALCOHOL DEHYDROGENASE ACTIVITY IN EXPERIMENT 2

Diet	Alcohol Dehydrogenase Activity (mmol hr^{-1} liver)
BS-711 (C)	2.88 ± 0.18
BS-711 (E)	2.41 ± 0.09
SBS-711 (C)	2.95 ± 0.13
SBS-711 (E)	3.24 ± 0.16
WB (C)	3.18 ± 0.15
WB (Free-Choice)	2.93 ± 0.12

derived from outbred Wistar rats [7], we also measured these parameters in a group of Wistar female rats.

Figure 1 indicates that the growth curves of the P and Wistar female rats were similar. Figure 2 depicts the change in AER as a function of age in the P and Wistar female rats, when AER was expressed either as mmol of ethanol eliminated per hour per rat (left panel) or as mmol of ethanol eliminated per hour per kg body weight (right panel). When expressed as rate per rat, AER increased rapidly from 26-days of age to about 60-days and it remained constant thereafter. By comparison, AER expressed as rate per kg body weight declined sigmoidally with age and it reached a constant low only when the rats were about 120- to 180-days old. The patterns of change were similar in the P and Wistar female rats, with the curves for the Wistar rats slightly displaced to the right.

Experiment 2

In this experiment, the P rats were given different dietary regimens: groups 1 and 2 were pair-fed the ethanol and control BS-711 diet; groups 3 and 4 were pair-fed the ethanol and control SBS-711 diet; group 5 was given solid food and water ad lib and group 6 was given the solid food ad lib and the free-choice of 10 percent (v/v) ethanol and water. Table 2 summarizes the mean daily caloric intake of the P rats receiving the different dietary regimens. In the first 4 weeks of feeding, the daily caloric intake of the rats placed on the BS-711 diets was about 15 percent lower than the other groups. More importantly, owing to the low protein content of the BS-711 diets, the protein caloric intake throughout the 7-week period was only one-half of that consumed by the other groups. The daily caloric intake as ethanol in the SBS-711 group tended (not statistically significant) to be lower than that in the BS-711 group. By comparison, calorie consumption as ethanol in the free-choice group was clearly lower (12 to 29 percent) than that in the BS-711 group (at least for weeks 1 and 7).

Table 3 shows the effect of the different dietary regimens on ethanol intake expressed as g of ethanol kg^{-1} per day. Partly owing to the higher daily ethanol intake per rat (cf. Table 2) and to the lesser weight gain of the rats on the BS-711 diet (Table 4), ethanol intake expressed as g kg^{-1} per day was the highest in the BS-711 group.

Table 4 shows the effect of the different dietary regimens on body weight and AER. Rats on the BS-711 diet failed to gain weight in the 7-week study. By comparison, rats in all the other groups gained at least 40 g over this period. AERs were expressed both as mmol hr^{-1} per rat and mmol hr^{-1} per

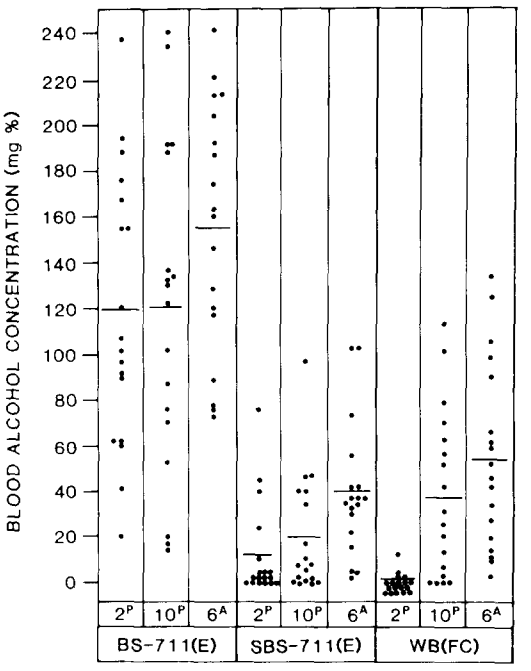

FIG. 3. Blood alcohol concentrations of P rats placed on three different dietary regimens. Horizontal bar indicates the mean value. BS-711(E), SBS-711(E) and WB(FC) are abbreviations for the ethanol-Bio-Serv-711 diet, the supplemented ethanol-Bio-Serv-711 diet and the Wayne-Blox free-choice drinking regimen.

kg body weight. Before initiation of the feeding experiment, the mean AERs of the 6 groups of rats were similar regardless of the manner of expressing the AERs: the mean AERs varied from 1.90 to 2.11 mmol hr^{-1} per rat and from 9.25 and 9.71 nmol hr^{-1} per kg body weight. By the end of the 7-week experiment, the AERs of all the groups treated with alcohol were 19 to 29 percent higher than their respective controls indicating that chronic alcohol ingestion, regardless of how ethanol was consumed, produced metabolic tolerance in the P rats. However, a real increase in AER over the 7-week experiment was evident in only 2 groups of animals, when AERs were expressed as mmol hr^{-1} per rat, i.e., those placed on the ethanol-SBS-711 diet and those given solid food and the free-choice drinking of 10 percent ethanol versus water. Except for the group of rats given solid food and free-choice drinking, all other groups exhibited an actual, net decline in AER at the end of the 7-week period when AER was calculated as mmol hr^{-1} per kg body weight. These data, therefore, demonstrate that: (1) The P rats given solid food and the free-choice drinking of 10 percent ethanol and water develop metabolic tolerance; (2) the BS-711 diet, unless supplemented with protein, will not produce a net increase in AER in ethanol-fed animals; and (3) interpretation of data with AERs expressed as mmol of ethanol eliminated per hr per rat is less complicated than when AERs are expressed as mmol hr^{-1} per kg body weight. This is due to the fact that, in female Wistar and P rats, the gain in body weight and the increase in AER as they mature from 50 to 200 days of age follow different time courses.

Table 5 summarizes the effect of the different dietary regimens on ADH activity in liver. In agreement with the bulk of the literature [2, 9–11, 24, 26], chronic alcohol treatment did not lead to a discernible increase in the total ADH activity.

TABLE 6

MEAN DAILY CALORIC INTAKE IN THE P RATS FORCED-FED ALCOHOL BY THE AIN DIET OR GIVEN SOLID FOOD AND THE FREE-CHOICE DRINKING OF ALCOHOL

	Mean daily caloric intakes per rat											
	Week 1			Week 2			Week 4			Week 6		
Total	Pr	E	Total	Pr	E	Total	Pr	E	Total	Pr	E	
AIN (C)	53.3	14.9		52.2	14.6	—	54.2	15.2	—	48.1	13.5	—
AIN (E)	54.1	15.1	19.2	53.9	15.1	19.1	54.1	15.1	19.2	48.3	13.5	17.1
WB (C)	59.4	14.6	—	54.5	12.7*	—	57.8	12.7*	—	46.7	10.4*	—
WB (Free-choice, water vs. 10 percent E)	61.2	15.2	13.3*	58.9	14.1	14.3*	64.0	14.7	17.7*	49.1	11.4*	14.6*

Abbreviations: C, control; E, ethanol; Pr, protein.
*Significantly different from either the AIN (C) or the AIN (E) diet (by analysis of variance).

TABLE 7

EFFECT OF ETHANOL GIVEN IN THE AIN LIQUID DIET AND BY FREE-CHOICE ON BODY WEIGHT AND ALCOHOL ELIMINATION RATE (EXPERIMENT 3)

	Body Weight g		Alcohol Elimination Rate mmol/hr/rat	
Diet	Before	6th Week	Before	6th Week
AIN (C)	178 ± 6	230 ± 5	1.78 ± 0.09	1.71 ± 0.09
AIN (E)	176 ± 6	246 ± 6	1.74 ± 0.08	2.09 ± 0.07
WB (C)	166 ± 5	244 ± 3	1.67 ± 0.09	2.03 ± 0.05
WB (Free-choice)	166 ± 5	239 ± 4	1.68 ± 0.10	2.36 ± 0.08

Brackets indicate that the compared values are significantly different.
Abbreviations: C, control; E, ethanol.

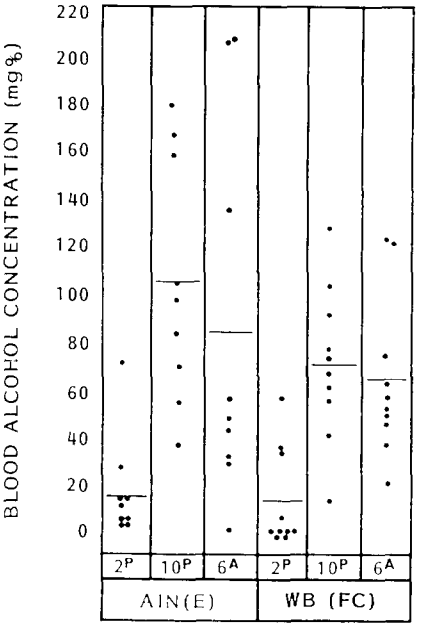

FIG. 4. Blood alcohol concentrations of P rats placed on the ethanol-AIN diet and the Wayne-Blox free-choice drinking regimen.

Figure 3 compares the blood alcohol concentrations of the P rats receiving alcohol with the different dietary regimens. Only those on the ethanol-BS-711 diet exhibited persistently elevated blood alcohol concentrations when measured at 6 a.m., 2 p.m. and 10 p.m. The animals given the higher protein diets, i.e., the SBS-711 or the WB diet, exhibited much lower blood alcohol concentrations and maintained a diurnal cycling of blood alcohol concentrations. In P rats given free-choice drinking of alcohol, blood alcohol concentrations ranged from 5 mg percent to as high as 137 mg percent during the dark cycle at the times of measurement.

Experiment 3

The AIN liquid diet is a formulation that has a higher protein content than the BS-711 diet (Table 1). Table 6 compares the mean total daily caloric intake per rat and the mean contribution of protein and ethanol to the total daily caloric intake. With forced feeding of ethanol (i.e., the AIN liquid diet with 6 percent ethanol), the daily intake of ethanol per rat was initially 44 percent (week 1) and then 8 and 17 percent (weeks 4 and 6) higher than that with free-choice drinking of ethanol (i.e., WB and free-choice drinking of 10 percent ethanol vs. water). When the alcohol intake was expressed as g/kg/day, forced-feeding of ethanol as part of the AIN liquid diet also led to initially 40 percent (week 1) and then 15 percent (week 6) higher alcohol intake than that with free-choice drinking. The daily alcohol intake of the P rats on the ethanol-AIN liquid diet and those on free-choice alcohol intake ranged from 10.1 to 13.8 g/kg/day and from 8.8 to 11.5 g/kg/day, respectively. Table 7 shows the amount of weight gain and the change in AERs as a function of time in the P rats pair-fed the AIN liquid diets (with or without ethanol) and in those pair-fed the Wayne-Blox solid laboratory food and 10 percent ethanol or an isocaloric amount of a sucrose solution. Weight gain of 29 to 47 percent was evident in all the groups over the 6-week period. AERs, expressed as

mmol hr $^{-1}$ per rat, increased over the 6-week period in the AIN(E), WB(C) and the WB (free-choice) groups. Metabolic tolerance was demonstrated in the P rats given ethanol either by forced-feeding or by free-choice. The mean increases in AERs when the P rats were forced-fed and when they were given alcohol by free-choice were 22 and 16 percent, respectively.

Figure 4 depicts the blood alcohol concentrations of the P rats when they were on the ethanol-AIN liquid diet and when they were given ethanol by free-choice. Both groups exhibited diurnal cycling of alcohol consumption, which was reflected in the diurnal variations of blood alcohol concentrations. With free-choice drinking, the blood alcohol concentrations in the P rats ranged from 17 to 127 mg percent during the dark cycle and they were similar to those of the P rats given the ethanol-AIN diet.

DISCUSSION

Recent studies [7, 19, 27] of the selectively-bred P and NP rats indicate that the P line of rats satisfies almost all of the perceived requirements for an animal model of alcoholism and that the P and NP lines are relevant models to study the biochemical, physiological and behavioral concomitants of alcohol drinking behavior. The data presented above clearly show the development of metabolic tolerance in the P rats following the free-choice drinking of ethanol for 6 to 7 weeks. In two separate experiments (Tables 4 and 7), AERs, expressed as mmol/hr/rat, increased 18–40 percent when compared with values before alcohol exposure and increased 16–20 percent when compared with values from the pair-fed and ad lib controls. In one experiment, the controls were free-fed, whereas in the other, the controls were pair-fed an amount of sucrose solution that is isocaloric to the amount of ethanol consumed by the experiment group. These findings are not unexpected because Hawkins *et al.* [3] have reported earlier that chronic alcohol ingestion of 8 g/kg/day can produce metabolic tolerance *in vivo* in the rats. Additionally, Videla *et al.* [25] have reported that liver slices from rats exposed to alcohol in the range of 3 to 8 g/kg/day exhibited enhanced alcohol metabolic rates *in vitro*. Since the female P rats consumed 7–12 g of ethanol/kg/day during free-choice drinking (Tables 3 and 7), metabolic tolerance should have developed and it did.

The biochemical basis of the development metabolic tolerance in rats remains controversial. An increase in ethanol oxidation rate mediated by induction of the microsomal ethanol-oxidizing system (MEOS) has been proposed as one mechanism [8,9]. An increase in ethanol oxidation catalyzed by the ADH pathway has also been advocated as another [5, 15, 20, 23]. Although some investigators [1,17] have reported an increase in the actual amount of ADH in liver when rats were exposed to alcohol chronically, most [2, 9–11, 24, 26] have found no change. No change was observed in the P rats in this study (Table 5). At the present, an increase in ethanol oxidation mediated by the ADH pathway after chronic exposure to alcohol is best explained by enhanced turnover of NADH. The latter can arise from increased rate of mitochondrial oxidation of NADH and/or from an increased rate of transhydrogenation and NADPH turnover due to induction of MEOS.

In most of the previous studies of metabolic tolerance in rats, alcohol elimination was compared using simply plasma ethanol disappearance rates (mg ethanol/100 ml plasma per hr) or AERs calculated and expressed as mmol hr^{-1} per kg body weight [3, 10, 11, 15, 20, 24, 26]. Plasma ethanol disappearance rate is not a good basis for comparison because it does not take into account changes in the volume of distribution of ethanol. Expressing the AER as mmol of ethanol eliminated hr $^{-1}$ per kg is probably also less than ideal. This is because the ethanol oxidizing capacity located primarily in the liver may bear no relationship or at best, a complex relationship, with body weight. As shown by Fig. 2, when AERs expressed as mmol hr^{-1} per kg are plotted as a function of age, they decreased sigmoidally and became constant only at 120 to 180 days of age. By comparison, when AERs are expressed as mmol hr^{-1} per rat, the relationship of AERs with age increases curvilinearly and becomes constant in early adulthood. Thus, as long as the rats at the beginning of the experiment are of the same age and body weight, the effect of any variable on AERs as a function of time can be more confidently interpreted if AERs are calculated as mmol hr^{-1} per rat. Of note, Israel *et al.* [4] have reported earlier a sigmoidal decline in AERs with growth, calculated as mmol hr $^{-1}$ per kg body weight, in male Spontaneous Hypertensive and male Wistar rats. The data shown here in Fig. 2 indicates that a similar curve obtains in female Wistar and P rats.

Previous studies on metabolic tolerance in rats also have not compared AERs before and after alcohol exposure. The present study examined specifically this relationship and assessed the effect of different dietary regimens. The development of metabolic tolerance to alcohol could be demonstrated with the feeding alcohol with any of the dietary regimens used by comparing the AERs of alcohol-fed rats with their concomitant controls at the end of the feeding experiment. However, a net increase through comparison of AERs before and after alcohol exposure was evident only in the P rats fed the alcohol-containing SBS-711 and AIN diets and in those given the solid food and alcohol by free-choice drinking (Tables 4 and 7). The lack of net increase in AER as a function time in the P rats fed the ethanol containing BS-711 diet is best explained by the low protein content of this diet (Table 2). The low dietary protein content is also most likely responsible for the disruption of the normal diurnal cycling of alcohol ingestion and the persistently elevated blood alcohol concentrations in the P rats given alcohol in the BS-711 diet (Fig. 3). With all the other dietary regimens wherein the protein intake was higher, food and alcohol consumption followed a diurnal rhythm. Accordingly, blood ethanol concentrations also cycled in a diurnal manner, i.e., they were low during the light cycle and high in the dark cycle. These results are in accord with those reported earlier by Rogers *et al.* [22]. Since the P rats developed metabolic tolerance when they were fed alcohol with diets which did not produce persistently elevated blood alcohol concentrations, it is clear the persistent intoxication is not a necessary condition to metabolic tolerance development.

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