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Effect of Addition of Ethanol and NaCl on Saccharin + Glucose Polydipsia[1]

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KULKOSKY, P. J. *Effect of addition of ethanol and NaCl on saccharin + glucose polydipsia.* PHARMAC. BIOCHEM. BEHAV. 10(2) 277–283, 1979.—Rats received an ad lib choice of food, water, and a solution containing saccharin, glucose, and NaCl solutes either as single stimuli or in combinations. Ethanol was gradually added to these vehicles or water from 0.5–15% w/v. Ethanol intakes of all groups with vehicles containing glucose were higher than intakes of the water vehicle group. Ethanol intakes of the 0.125% saccharin+3.0% glucose+1.0% NaCl+ethanol group were highest, peaking at >9.0 g/kg/day, and this group displayed the highest blood ethanol levels. However, there was no evidence of withdrawal syndrome, nor of increased intake of unflavored ethanol by groups previously receiving flavored ethanol. It is suggested that ethanol eliminative capacity limits free-choice ethanol intake when maximized by the addition of sapid congeners.

Saccharin Glucose NaCl Saccharin and glucose polydipsia Ethanol intake Blood ethanol Alcohol withdrawal

SUBSTANTIAL effort has been devoted to development of procedures to increase ethanol intake by animals [3, 23, 26, 28, 32, 35, 44, 45, 46]. The goal of these efforts is to provide a valid animal model of the human conditions of alcohol abuse and alcoholism. Such an experimental model should greatly aid research directed to understanding, treatment and prevention of alcohol-related disorders in humans [24].

An application of Falk's schedule-induced polydipsia paradigm succeeds in production of sustained high daily ethanol intakes and blood levels, and alcohol withdrawal symptoms when ethanol is removed [9]. This technique first induces greatly increased daily water intake by the presentation, with a specified intermittency, of small dry food pellets to hungry, weight-reduced rats that have water continuously available. Once polydipsia is established, ethanol is gradually added to the available fluid in concentrations increasing from 1–6% v/v. The results of this procedure meet many of the requirements for an animal model of alcoholism, but the method does not satisfy all the criteria for an ideal volitional model as defined by Lester and Freed [24] because food and water access is necessarily restricted, and body weight is initially reduced to 80% of the free-feeding level.

Valenstein, Cox and Kakolewski [42] reported another form of polydipsia in the rat that requires neither food restriction nor body weight reduction. They showed that rats will consume extremely large volumes of a 3% glucose and 0.125 (or 0.25)% sodium saccharin solution presented ad lib along with lab chow and an alternate fluid choice of either water, 3% glucose or 0.25% sodium saccharin. Daily intakes of the saccharin plus glucose solutions averaged between 130 and 190 ml/rat, and occasionally exceeded the individual rat's body weight. Only very low amounts of the alternate fluids were consumed. Although this is a robust, simple and often-replicated [41] method for inducing polydipsia in a variety of rodents, the effect of addition of ethanol to this saccharin+glucose solution has received but limited attention.

Geiger and Barker [14] reported a large increase in intake of 10% ethanol after presentation for a single day in a 4% sucrose + 0.25% sodium saccharin solution. Also, Geiger [13] observed that rats ingest many times more ethanol from 3, 6, 12, or 24% ethanol + 4% sucrose + 0.25% sodium saccharin solutions than from unsweetened ethanol solutions at these concentrations.

In the following experiment, ethanol was gradually added to saccharin, glucose and NaCl solutions, according to a procedure similar to that used in schedule-induced ethanol polydipsia. Solutions containing both single solutes and combinations of solutes were tested as dipsogenic vehicles for the optimization of ethanol intake. An ad lib choice of water and food was also continuously present. Sodium chloride was examined in addition to saccharin and glucose because added or administered NaCl has been reported to result in increased ethanol solution intake [10, 19, 43] and

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TABLE 1
SEQUENCE OF TREATMENTS

	Days					
	1–5	6–20	21–35	36–40	41	42–56
Fluids Available:	All groups: H_2O only	All groups: H_2O and vehicles	"+E" groups: choice of H_2O and vehicles with ethanol added from 0.5–15%; other groups: choice of H_2O and vehicles	"+E" groups: choice of H_2O and vehicles with 2.5% ethanol added; other groups: choice of H_2O and vehicles	All groups: H_2O only	All groups: choice of H_2O and ethanol/water solution from 0.5–15%

tolerance [40]. Fluid and absolute ethanol intakes, blood ethanol levels, withdrawal, and subsequent intakes of unflavored ethanol solutions induced by availability of the various solutions were investigated.

METHOD

Animals

Animals were 90 experimentally naive male Charles River outbred Wistar descended rats (Crl: COBS (WI) BR), approximately 5 weeks of age at housing. Each rat was individually housed in a wire mesh stainless steel cage at an ambient temperature of 20°C and 12:12 L:D (8 a.m.–8 p.m. light) lighting cycle, and had ad lib access to Purina Rat Chow and deionized water throughout the experiment.

Apparatus

Two hundred fifty and 100 ml calibrated drinking tubes fitted with valveless stainless steel spouts were used to measure fluid consumption to the nearest 1.0 ml. Spillage from drinking tubes was caught by 2 oz jars fitted with 60° funnels and positioned under the cages. Food intakes from stainless steel hoppers, and body weights were measured to the nearest 1.0 g.

Procedure

Each rat was individually housed and randomly assigned to one of 18 treatment groups. All groups received ad lib access to deionized water as sole fluid for an initial period of 5 days. Throughout the experiment, fluid intakes were measured daily. Body weights were recorded every 5 days. For a total of 15 days after this initial 5-day period, rats received either ad lib water as sole fluid (Groups H and E), or a choice of water and a solution of deionized water and the following solutes and concentrations: 0.125% w/v Na saccharin (Fisher purified, "S"); 3.0% w/v glucose (anhydrous dextrose, Baker reagent, "G-3"); 9.0% w/v glucose ("G-9"); or 1.0% w/v NaCl (Baker reagent, "N"). The solutions contained either single solutes (Groups S, G-3, G-9, N, S+E, G–3+E, G–9+E, N+E), or combinations of solutes (Groups S+G–3, S+N, G–3+N, S+G–3+N, S+G–3+E, S+N+E, G–3+N+E, S+G–3+N+E). Relative positions of fluids were alternated daily. On the 14th day of this period, food intakes of all groups were measured and corrected for spillage. Following this 15-day period, rats in Groups E, S+E, G–3+E, G–9+E, N+E, S+G–3+E, S+N+E, G–3+N+E, and S+G–3+N+E received a choice of water and the corresponding solution (or water) with ethanol (from U.S.P. 95%, "E") added according to the following concentration sequence: 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, 7.0, 9.0, 12.0, 15.0% w/v, increasing one concentration per day. Rats in the remaining groups continued to receive a choice of water (or water only) and the appropriate solution without addition of ethanol.

After completion of the ethanol concentration sequence, each ethanol-receiving group was then given a choice of its vehicle with 2.5% w/v ethanol added and water for a period of 5 days. Tail blood samples were taken from rats in Groups H, E, G–9+E and S+G–3+N+E at 12 noon and midnight of the 4th day, and assayed for ethanol content by enzymatic technique [6]. Other groups continued to receive either the appropriate sapid solution without ethanol and water, or water only. On the fifth day of this period, rats in Groups H, E, G–9+E, and S+G–3+N+E were placed in a 200×15×15 cm runway of painted wood with a hinged Plexiglas top, as described by Pohorecky [33]. The rats were placed at one end of the runway delimited by a line 25.4 cm (10.0 in.) from the wall, and the time to complete the first run (defined as a crossing of the corresponding line at the other end), and the total number of runs made in 5 min were recorded. After this test, all rats were given only food and water. At the same time on the following day, rats in the above groups were again tested in the runway. Pohorecky [33] has shown that rats maintained on an all liquid+ethanol diet and undergoing ethanol withdrawal display greatly decreased locomotor activity in this test compared to controls, as indexed by number of runs per trial and time to complete the first run. Immediately following the second runway test, all rats in all groups then began to receive a choice of water and an ethanol/water solution presented in the same ascending concentration sequence as described above. The sequence of treatments is summarized in Table 1.

Data were analyzed with 1- and 2-way analyses of variance, with $p<0.05$ as significant.

RESULTS

Mean body weights of the 18 groups did not differ signifi-

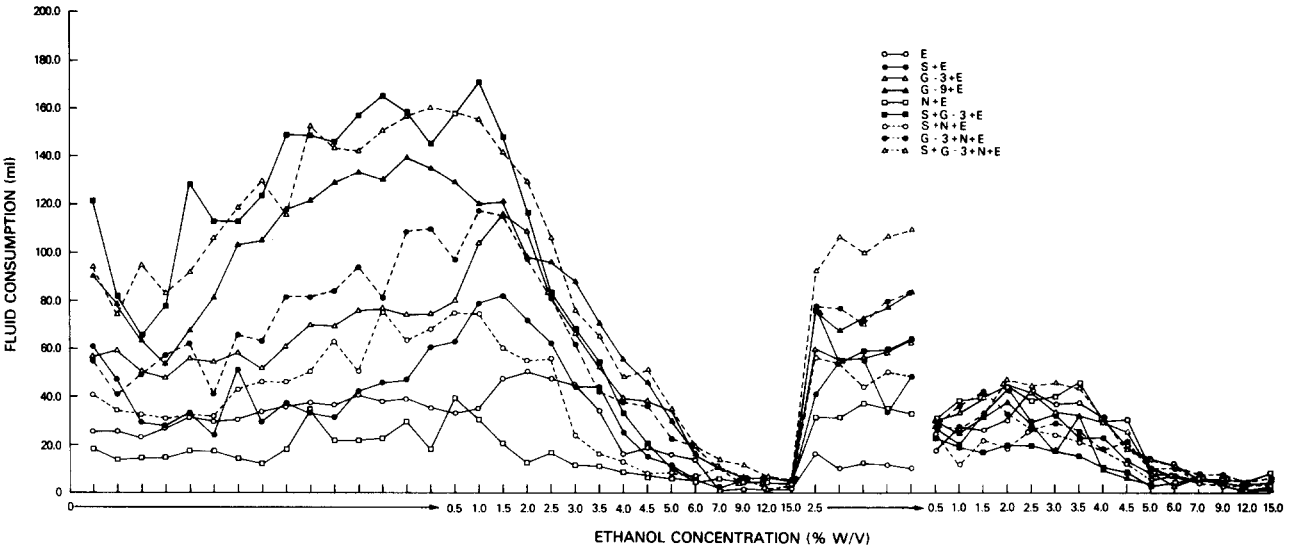

FIG. 1. Mean vehicle (+ethanol) intakes (in ml) of Groups E (water vehicle), S+E (0.125% Na saccharin vehicle), G–3+E (3.0% glucose vehicle), G–9+E (9.0% glucose vehicle), N+E (1.0% NaCl vehicle), S+G–3+E (0.125% Na saccharin+3.0% glucose vehicle), S+N+E (0.125% Na saccharin+1.0% NaCl vehicle), G–3+N+E (3.0% glucose+1.0% NaCl vehicle), and S+G–3+N+E (0.125% Na saccharin+3.0% glucose+1.0% NaCl vehicle) as a function of ethanol concentration (in % w/v). During period of 0.0% ethanol, all groups have a 2-bottle choice of vehicles (intakes displayed) and water except Group E (one-bottle water intake displayed). During the first sequence of ethanol addition and 2.5% ethanol addition, all groups have a 2-bottle choice of vehicles+ethanol (intakes displayed) and water (ethanol/water solution intakes displayed for Group E). During final sequence of ethanol addition, all groups have a 2-bottle choice of only ethanol/water solution (intakes displayed) and water.

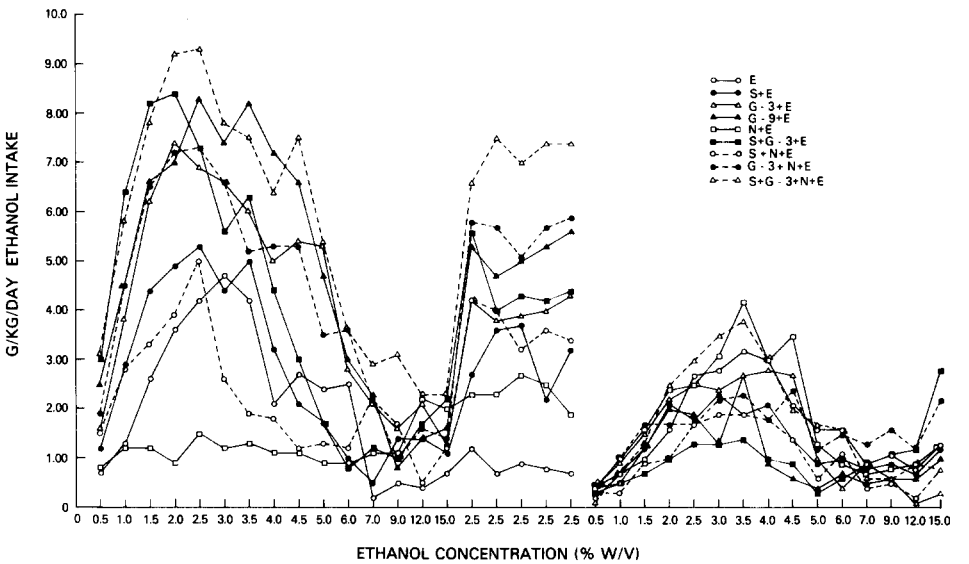

FIG. 2. Mean grams per kilogram of body weight per day intakes of absolute ethanol of Groups E, S+E, G–3+E, G–9+E, N+E, S+G–3+E, S+N+E, G–3+N+E, and S+G–3+N+E as a function of ethanol concentration (in % w/v). Legend and fluid choice conditions as in Fig. 1.

cantly at any measurement point of the experiment, all Fs(17,72)<1.0, $p>0.05$. Grand mean body weight increased from 108.6 g at Day 1 to 425.6 g at Day 56. Although mean food intakes of all groups differed significantly on Day 19 of the experiment, F(17,72)=6.5, $p<0.05$, mean caloric intakes did not, F(17,72)=0.7, $p>0.05$.

Rats in Groups S+G–3+E, S+G–3+N+E, and G–9+E rapidly developed polydipsia (means approx. 120–170 ml/day) during the initial 15 day period of solution availability. Solution intakes of these groups rapidly declined at added ethanol concentrations >2.0%, and water intakes increased. Vehicle intakes of the remaining groups receiving vehicle+ethanol treatment were substantially lower during the initial 15 day habituation period, but these groups typically exhibited the same pattern of declining solution intakes and increasing water intakes with increasing ethanol concentration. However, transient increases in solution intakes were observed in Groups G–3+E and S+E at 0.5–2.0%

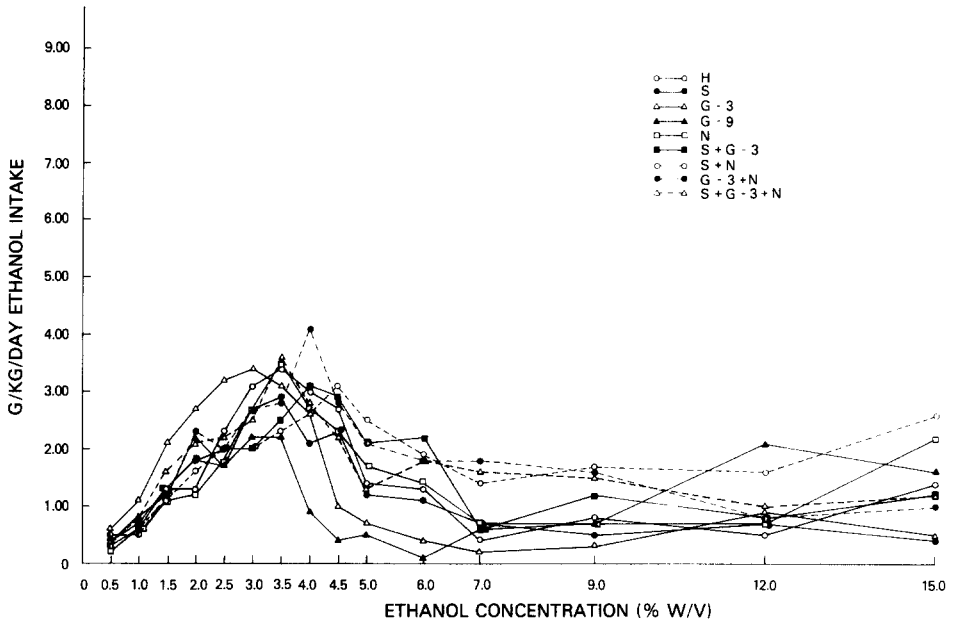

FIG. 3. Mean grams per kilogram of body weight per day intakes of absolute ethanol of Groups H (prior water only), S (prior choice of 0.125% Na saccharin and water), G–3 (prior choice of 3.0% glucose and water), G–9 (prior choice of 9.0% glucose and water), N (prior choice of 1.0% NaCl and water), S+G–3 (prior choice of 0.125% Na saccharin+3.0% glucose and water), S+N (prior choice of 0.125% Na saccharin+1.0% NaCl and water), G–3+N (prior choice of 3.0% glucose+1.0% NaCl and water), and S+G–3+N (prior choice of 0.125% Na saccharin+3.0% glucose+1.0% NaCl and water) as a function of ethanol concentration (in % w/v). All groups have a 2-bottle choice of only ethanol/water solution and water during this period.

ethanol. Group E consumed more ethanol/water solution than water at ethanol concentrations from 0.5–3.5%. Solution intakes of the 9 vehicle+ethanol groups are depicted in Fig. 1.

Mean grams of absolute ethanol per kilogram of body weight per day intakes of Groups E, S+E, G–3+E, G–9+E, N+E, S+G–3+E, S+N+E, G–3+N+E, and S+G–3+N+E across the three phases of ethanol addition are shown in Fig. 2. Mean g/kg/day intakes of ethanol of Groups H, S, G–3, G–9, N, S+G–3, S+N, G–3+N, and S+G–3+N across the phase of choice between water and ethanol/water solution are shown in Fig. 3.

Across the initial period of vehicle+ethanol availability, mean g/kg/day intakes of the 9 groups differed significantly, $F(8,540)=43.8$, $p<0.05$, and showed significant variations with concentration, $F(14,540)=37.4$, $p<0.05$; the interaction of groups and concentrations was also significant, $F(112,540)=1.8$, $p<0.05$. Mean intakes of Group E differed significantly from all groups except S+E and S+N+E. Mean intakes of Group S+G–3+N+E were highest, and differed significantly from all other groups. During the period of vehicle +2.5% ethanol availability, mean g/kg/day intakes of the 9 groups differed significantly, $F(8,180)=25.9$, $p<0.05$, but the effects of days, $F(4,180)=0.2$, $p>0.05$, and the interaction, $F(32,180)=0.3$, $p>0.05$ were not significant. Mean g/kg/day intakes of both Group E (lowest) and Group S+G–3+N+E (highest) differed significantly from each of the other groups across this period.

Mean blood ethanol levels of the 4 groups sampled differed significantly at midnight, $F(3,16)=5.6$, $p<0.05$, but not at noon, $F(3,16)=1.1$, $p>0.05$. Individual values and means of the groups at midnight and noon are shown in Table 2. At midnight mean blood ethanol level of Group S+G–3+N+E was highest and differed significantly from each of the other groups, but none of the other groups differed significantly from each other.

In the runway test, the 4 groups showed no significant differences in either pre-post withdrawal changes in number of runs made in 5 min, $F(3,16)=0.03$, $p>0.05$, or in mean pre-post withdrawal time to complete first run, $F(3,16)=0.9$, $p>0.05$.

Across the final 15 day period of ethanol/water and water choice, mean g/kg/day intakes of the 18 groups showed small, but significant differences, $F(17,1080)=4.2$, $p<0.05$. The effect of concentration was significant, $F(14,1080)=32.3$, $p<0.05$, but the interaction was not, $F(238,1080)=0.9$, $p>0.05$. Intakes of all groups not receiving ethanol previously were significantly greater than intakes of all groups previously receiving ethanol, $F(1,1080)=8.9$, $p<0.05$.

DISCUSSION

The data clearly show that gradual addition of ethanol to certain sapid vehicles containing glucose results in ethanol intake greatly augmented beyond that observed with a water vehicle. Addition of ethanol at optimal concentration to the most effective vehicle, a combination of saccharin and glucose [42] and sodium chloride, results in ethanol intakes within the lower range attained in techniques designed to produce physical dependency on alcohol [28]. With this solution, mean blood ethanol levels observed at midnight are higher than means previously reported for rats in self-selection experiments [25], experiments on genetic selection for alcohol consumption [8], and studies of shock-induced

TABLE 2

BLOOD ETHANOL LEVELS (MG/DL) OF GROUPS H, E, G-9+E, AND S+G-3+N+E AT MIDNIGHT AND NOON

Group	S	Midnight	Noon
H	211	0.0	2.7
	213	0.0	0.0
	236	0.0	2.0
	250	1.0	5.8
	297	5.0	0.0
	Mean	1.2	2.1
E	229	0.0	0.0
	259	0.0	3.6
	270	4.2	0.0
	275	0.0	4.2
	277	0.0	0.0
	Mean	0.8	1.6
G-9+E	209	7.0	3.8
	220	3.5	0.0
	224	4.0	0.0
	268	14.2	0.0
	283	11.8	0.0
	Mean	8.1	0.8
S+G-3+N+E	210	52.2	4.0
	233	28.1	0.0
	249	94.6	46.5
	265	4.2	3.3
	272	22.0	0.2
	Mean	40.2	10.8

ethanol consumption [29]. However, these blood levels are only within the lower range of levels produced with schedule-induced ethanol polydipsia [9] and other techniques that successfully induce alcohol withdrawal syndrome [28]. Values observed at noon indicate that these blood levels are too low, and fluctuate too widely to result in unequivocal signs of physical dependence, as measured in a runway test [33,39].

Data obtained from the period of choice between water and unflavored ethanol solution indicate that the high levels of intake produced with sapid vehicles are not accompanied by incrased intake of unflavored ethanol. Actually, many groups previously receiving ethanol showed a lower intake of unflavored ethanol than their control groups previously naive to ethanol (the saccharin+glucose+NaCl+ethanol group was a slight exception). This finding is in accord with the "contrast effect" of Cullen, Croes and Gillis [5] of lower unflavored ethanol intake after previous experience with a sucrose+ethanol solution, relative to unexperienced controls.

Many previous studies have demonstrated an elevation of ethanol intake by addition of sapid solutes. The additional sapid congeners used have been saccharin [7, 15, 38, 43], sugars [5, 15, 16, 22, 36, 43], the combination of saccharin and sugar [13,14], sodium chloride [10, 19, 43], or compound stimuli of somewhat unspecified composition, e.g., commercially available alcoholic beverages [2,34], "Fanta" [31], and fruit juices [11,30]. Often a concentrated sugar solution vehicle is employed. In the present study, 9% glucose was not only less effective in elevation of ethanol intake and blood levels than 3% glucose+saccharin+NaCl, but also 9% glucose resulted in greater depression of food intake (G-9 mean=17.5 g; S+G-3+N mean=23.6 g; H_2O mean=28.0 g).

In the present study, all solutions containing glucose proved superior vehicles to water for optimizing ethanol intake. Electrophysiological [17] and psychophysical [27] evidence indicates that the combination of ethanol and sugar results in augmented gustatory nerve responses and ratings of sweetness, compared to sugar presented alone. Perhaps this effect underlies the observed initial increase in solution intake by Groups G-3+E and S+E at low concentrations of added ethanol.

All groups receiving ethanol declined in both solution intake and absolute ethanol intake, and increased water intake, with added ethanol concentrations beyond approximately 3.5%. A similar decline in intake with increasing ethanol concentration was reported by Holman and Myers [18] for schedule-induced ethanol polydipsia. This decline of intake may reflect increasing aversion to either the oronasal sensory or malaise-producing properties of ethanol. Reappearance of very similar solution intakes when 2.5% ethanol was re-presented suggests that the decline is due to oronasal sensory aversion—not conditioned aversion. Previous studies [20,35] have shown that olfactory inputs are essential to the rat's typical rejection of certain concentrations (approximately 5–16%) of ethanol. However, at high concentrations (approximately 16%), even anosmic rats reject ethanol solutions, which indicates the importance of non-olfactory sensory systems in mediation of rejection of high-concentration ethanol solutions. A conditioned aversion explanation might be invoked to account for the lower intakes of unflavored ethanol by some groups which had previously received flavored ethanol.

The peak absolute ethanol intakes observed in this experiment are close to the maximal daily ethanol metabolic rates reported for rats with a variety of techniques [44], and determined for this strain by in vivo and in vitro hepatocyte analysis [4]. It has been suggested that ethanol eliminative capacity correlates with, and may effectively limit rodent ethanol consumption in a free-choice situation [1, 12, 37]. Repeated ethanol consumption beyond ethanol eliminative capacity should result in blood ethanol levels sufficiently aversive to condition a taste aversion to the available ethanol solution. Thus, maximal ethanol eliminative capacity should limit free-choice ethanol consumption, as the present results suggest.

The rat's regulatory ability to avoid exceeding the maximal daily ethanol eliminative capacity by free-choice intake might be of crucial adaptive significance for a species whose forebears' diet may often include many fermenting foods (cf. [21]). Thus some degree of food or water restriction, such as employed in schedule-induced ethanol polydipsia, or physiological manipulation, might be necessary to achieve a rat model of alcohol addiction, however imperfect the resulting model may be. Nevertheless, in any model, as Meyers *et al.* have pointed out [30], use of an adequate vehicle for ethanol presentation is important. The present experiment describes one vehicle for optimization of ethanol intake without food

or water restriction, or physiological manipulation. Its use in other paradigms might facilitate production of high ethanol intakes and blood levels, and alcohol dependency and withdrawal syndrome. The saccharin+glucose+NaCl solution has also shown to be a very effective vehicle for achieving substantial self-administration of substances other than ethanol, e.g., vitamins, antibiotics, and other drugs (Kulkosky, unpublished observations). This vehicle might be employed when voluntary self-administration of experimental or therapeutic substances is a procedural advantage.

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