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Determinants of Ethanol Intake in Rats: Concurrent Access to Water and Side Positions of Water and Ethanol

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ABSTRACT

Water and ethanol solutions were concurrently made available to 4 food-deprived male albino rats during daily 1-hr sessions in an operant conditioning chamber equipped with two levers and two liquid dippers. The side positions of the two liquids were reversed from session to session, and each response was reinforced. The number of ethanol reinforcements substantially exceeded the number of water reinforcements for each rat at each concentration studied (8, 16 and 32% W/V) regardless of the side of ethanol availability. Number of ethanol reinforcements did not differ between sides. Water reinforcements were low in number and did not vary with ethanol concentration. As the ethanol concentration was increased, the number of ethanol reinforcements obtained decreased, while the quantity consumed (mg/100 g of body weight/hr) increased. The highest rate of responding occurred at the beginning of the session.

Intake of an ethanol solution may be due to its liquid character and not due to the presence of ethanol. To control for this possibility most animal studies of ethanol drinking have employed two drinking bottles -- one containing water and the other containing an ethanol solution (see Myers and Veale, 1972, and Wallgren and Barry, 1970, for recent reviews). Concurrent access to water, when combined with systematic changes in the relative positions of the different liquids, permits an assessment of the portion of ethanol consumption that may be attributed to nonspecific liquid intake.

Myers has made food, water, and ethanol concurrently available to rats, with the presentation of each being contingent upon a lever press (Myers and Carey, 1961; Myers, 1961a, 1961b). The rats were both food and liquid deprived. With this procedure he has studied the effects of a number of factors, including ethanol concentration on responding for each of the three substances. When the concentrations were presented in an ascending order, ethanol responses exceeded those for water until the ethanol concentration reached 9% (V/V); whereas when the concentrations were presented in a descending order, ethanol responses did not exceed those for water until the concentration was decreased to 4% (V/V) (Myers and Carey, 1961). The procedure used in the present study is similar to Myers', in that water and ethanol were concurrently available to food-deprived rats contingent upon lever pressing.

The purpose of this study was to investigate the effects of concurrent access to water on ethanol intake. Additionally, the quantity (mg) of ethanol consumed and the time course of intake were measured. The influence of side preference was evaluated by varying the location of water and ethanol. To simplify the experimental analysis, the rats were not liquid deprived and food pellets were not available in the operant chamber.

METHOD

Animals

Four male albino Sprague-Dawley rats, about 300 days old at the beginning of the experiment, were individually housed in a constantaly illuminated room with the temperature maintained at 24°C. At 80% of their free--feeding weights, at which they were maintained, the three rats weighed 477g (Z-7), 450g (Z-6) and 494g (Z-2). One rat, Z-9, was maintained at 403g which was 65% of its free-feeding weight. Water was always available in the rats' home cages, except for 5 days during training (see Procedure below).

Apparatus

A sound-attenuated commercial operant conditioning chamber (LVE, #1414) was equipped with two levers and two solenoid-driven liquid dippers (LVE, #1351). The levers and liquid dippers were symmetrically centered on the front panel with the dippers placed lateral to the levers. Three colored jewel lights located above each lever provided general illumination. A 4.76-W white light was located 3.2 cm above the hole in the panel where the dipper cup was located when in the up-available position. Each lever press produced 4-sec access to a dipper cup containing 0.12 ml of liquid. Simultaneous with the 4-sec dipper cup presentation was the sounding of a Sonalert and the illumination of the light above the dipper-panel opening. Liquid was contained in partially covered reservoirs to minimize evaporation. White masking noise was constantly present, and an exhaust fan provided ventilation.

Programming and data recording were automatically controlled by standard electromechanical equipment in an adjacent room. The temporal pattern of the responses and reinforcements was continuously recorded by a cumulative recorder and a counter which printed out every 2 minutes.

The ethanol concentrations, expressed in grams percent, were prepared using 95% (V/V) ethanol in tap water. For example, the 8% solution was made by adding 10.6 ml of ethanol to a volumetric flask with sufficient tap water to make a total volume of 100 ml. The solutions were prepared at least 20 hours before use and were kept in stoppered flasks at room temperature. The volume consumed was measured at the end of each session by subtracting the volume remaining from the volume added to the reservoir, corrected for evaporation.

Procedure

Establishment of lever pressing for water. Water bottles were removed from the rats' home cages, and to further increase the probability of drinking, the daily feedings of Purina Laboratory Chow were placed in a wire food hopper in the operant chamber. During the first daily 6-hr session, presentations of the right-hand dipper ocurred on a 1-min variable-time schedule. Such a schedule results in noncontingent dipper presentations occurring at a mean rate of once a min, but the time between particular presentations varies. After the rats learned to drink from the dipper, the variable-time water presentations were discontinued, and the rats were manually shaped to press the right-hand lever. After the rats learned to lever press, three more sessions were conducted before the water bottles were restored to the home cages.

Establishment of ethanol as a reinforcer. In-session feedings of Purina Laboratory Chow continued for a series of 14 daily 6-hr sessions. During the first 5 sessions water was the available liquid, then 2% ethanol for 2 sessions, 4% ethanol for 3 sessions, and 8% ethanol for 4 sessions. The in-session feedings were then discontinued, and the food was given to the rats only in their home cages following each session. Sessions with 8% ethanol available at the right dipper continued until 5 consecutive stable days of ethanol-reinforced lever pressing occurred. During this phase responses on the left-hand lever had no consequence.

Establishment of concurrent performance. After 8% ethanol was established as a reinforcer at the right dipper, availability of 8% ethanol was shifted to the left dipper until the rats emitted an approximately equal number of responses for ethanol in a given session at the left lever as they had in previous sessions at the right lever. Thereafter, 8% ethanol availability was shifted daily between the two dippers. Responding on the side opposite ethanol produced presentations of an empty dipper. After 10 consecutive sessions of stable ethanol-reinforced lever pressing (5 sessions on the left and 5 on the right), the session duration was reduced from 6 hr to 1 hr.

Experimental phase. After 10 consecutive 1-hr sessions of lever pressing for ethanol, concurrent water availability was introduced at the dipper opposite to the one containing ethanol. Intake of ethanol concentrations of 8, 16, 32 and 8% (retest), in that order, was studied under conditions of concurrent access to water. Changes from one concentration to the next were made after 10 sessions, given that there was no consistent upward or downward trend in the number of reinforcements. The positions of water and ethanol were systematically reversed from session to session except for the 8% retest series, where 8% was held on the right for 2 consecutive sessions and then on the left for 2 consecutive sessions.

RESULTS

Figure 1 shows that the number of ethanol reinforcements substantially exceeded the number of water reinforcements for each rat at each concentration regardless of the side of ethanol availability. For each rat at each concentration a *t*-test was made for the difference between the mean number of ethanol reinforcements obtained on each side. In no case was there a significant difference ($p < 0.05$, $df = 8$). Also, shown in Figure 1 are the mean numbers of reinforcements obtained when the rats were returned to 8% after completing the series of sessions at 32%. On retest at 8%, rat Z-9's and Z-6's reinforcements were decreased 25% and 3%, respectively, relative to the number initially obtained at 8%, whereas reinforcements for rats Z-2 and Z-7 showed increases of 35% and 3%, respectively. On retest with the 8% ethanol solution present for two consecutive days on one side and then for two consecutive days on the other side, the rats responded on the side where ethanol was available. Thus, the rats did not learn merely to alternate sides between sessions; the rats did discriminate the location of ethanol perhaps on the basis of odor (Meisch and Thompson, 1973).

Table 1 presents the group means for water and ethanol reinforcements on each side at each concentration. Dipper presentations on the side opposite ethanol were not increased by the addition of water (Table 1), and water reinforcements did not vary with ethanol concentration (Figure 1, Table 1). Moreover, the concurrent availability of water did not decrease the number of 8% ethanol reinforcements obtained (Table 1).

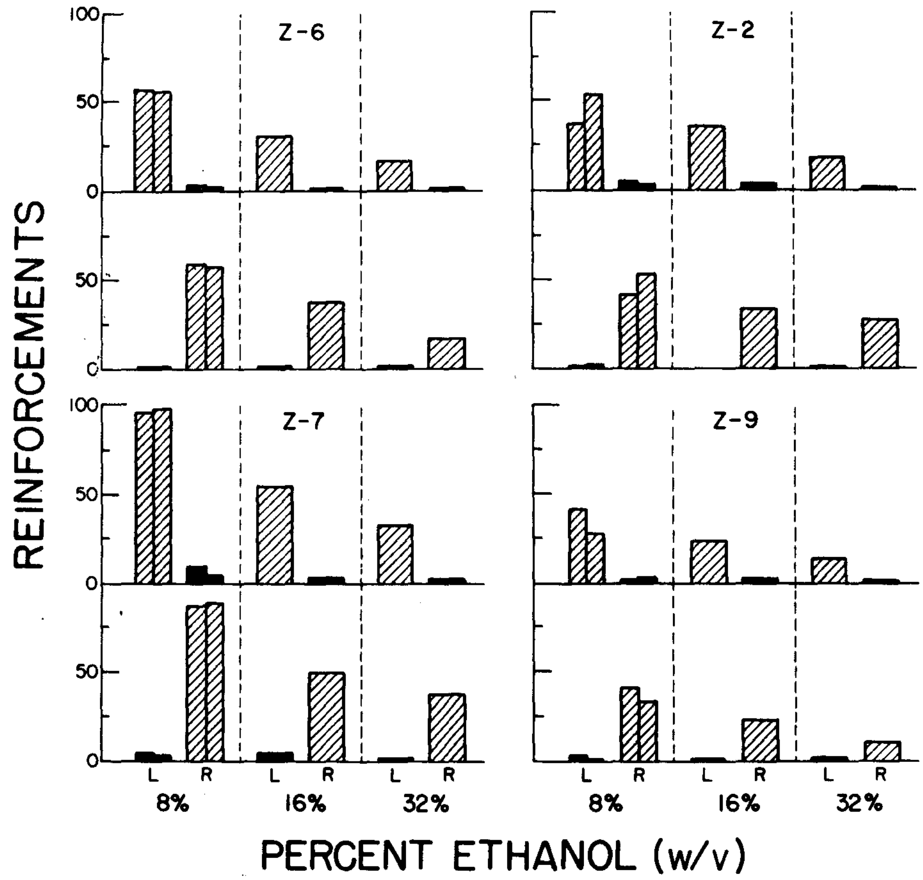

Figure 1. Ethanol and water reinforcements per 1-hr sessions as a function of ethanol concentration and position, left (L) or right (R) side. Striped bars: ethanol reinforcements. Solid bars: water reinforcements. Each bar represents the mean number of reinforcements obtained during 5 1-hr sessions. At 8%, where two pairs of bars are shown, the left-hand bar of each pair represents values obtained prior to the series of sessions at 16%, while the right-hand bar of each pair represents values obtained following the series of sessions at 32%.

As the ethanol concentration was increased, the number of reinforcements obtained decreased (Figure 1, Table 1). The decreases were not to a point below one-half that obtained at the adjacent lower concentration, and since the volume drunk was proportional to the number of reinforcements ob-

Table 1

Group Means

Ethanol Concentration W/V	Reinforcements		Quantity Consumed (mg/100 g body weight/hr)	
	Left	Right	Left	Right
E[†]	2.00**	7.90	-----	-----
8	48.90	45.45	116.2	108.0
0	1.50	4.75	-----	-----
8	57.40	57.10	120.7	120.0
0	0.95	2.45	-----	-----
16	35.80	34.35	150.4	144.6
0	0.95	1.75	-----	-----
32	20.10	20.65	169.2	173.8
0	1.00	3.60	-----	-----
8	58.55	58.40	123.3	123.0

E[†] = Empty dipper (no liquid present in reservoir).

n** = 20 (4 rats x 5 observations each)

tained, the quantity (mg) consumed increased with increases in the concentration (Figure 2).

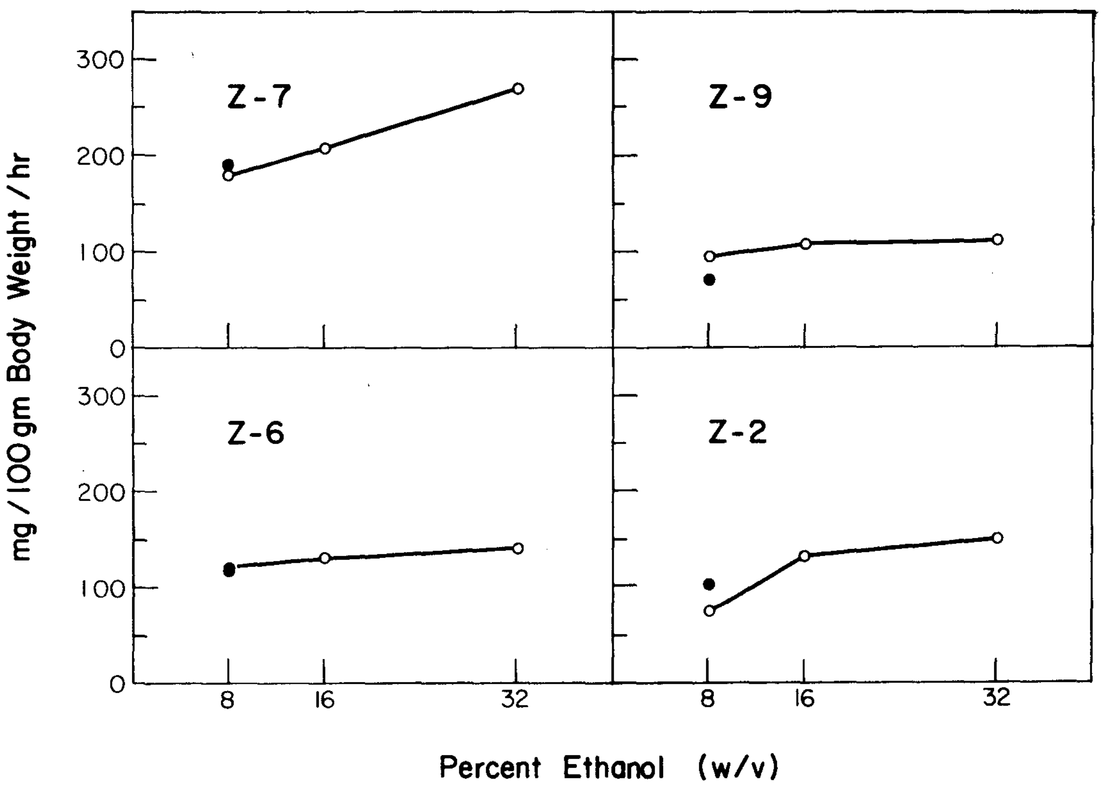

Figure 2. Quantity (mg/100 gm body weight/hr) of ethanol consumed per 1-hr session as a function of concentration. Each point is the mean value from 10 1-hr sessions. The filled circle at 8% represents the mean value of the series of sessions following those at 32%.

Figures 3 through 6 show that for each rat at each concentration most reinforcements were obtained at the beginning of each session. The time course of water intake was an exception: Reinforcements were low in number and were more evenly distributed throughout the session. Figure 7 shows that when responding occurred it was often in bursts.

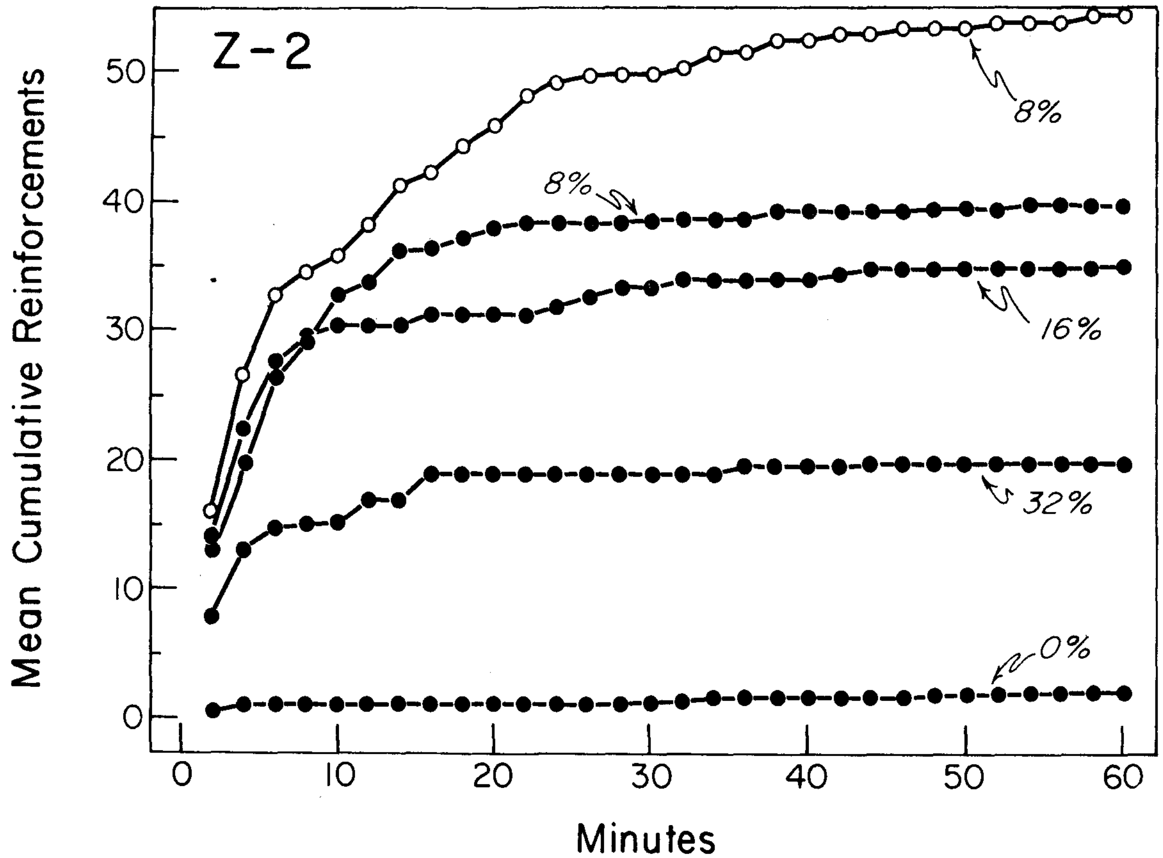

Figure 3. Rat Z-2's mean cumulative reinforcements over 1-hr sessions as a function of ethanol concentration. Each point is a mean based on observations from 10 sessions, except for those points at 0%, which are means from 40 sessions. Open circles represent values from sessions following the series of sessions at 32%.

DISCUSSION

Rats concurrently offered water and an ethanol solution chose the ethanol solution regardless of the side of ethanol availability. These results indicate that intake of the ethanol solutions was due to the presence of ethanol and not to their liquid character. It might be argued

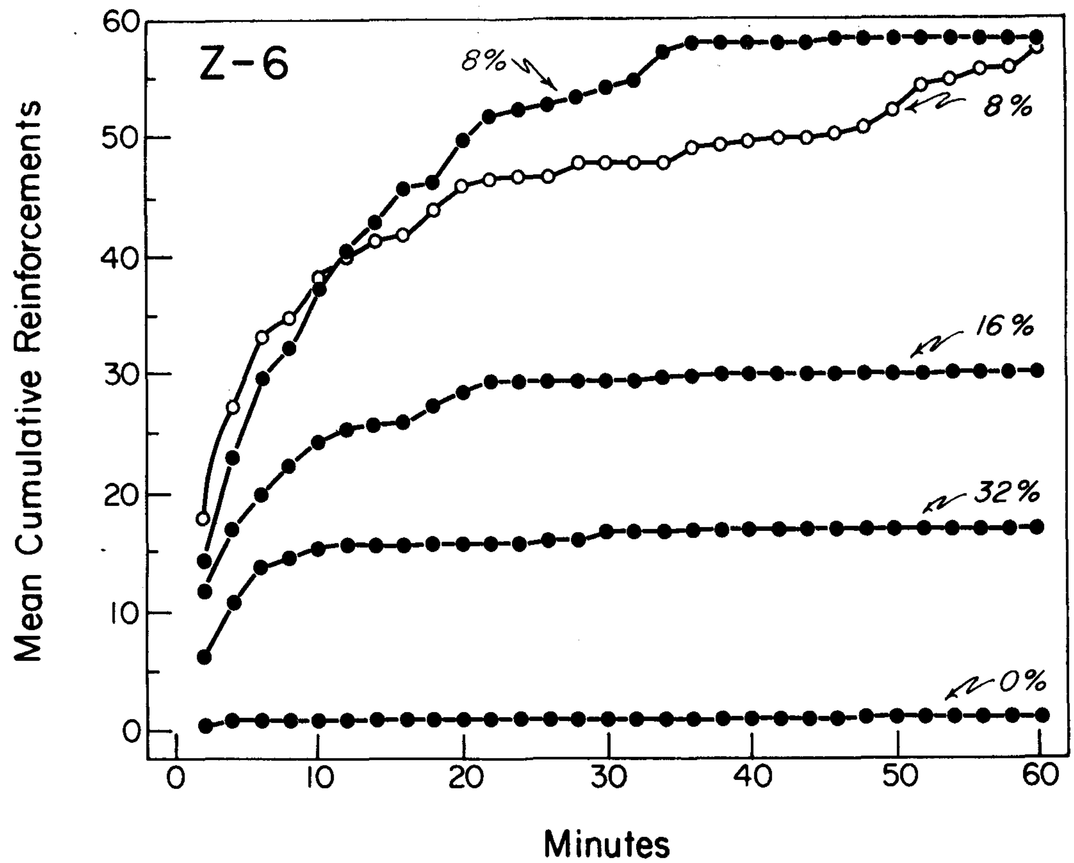

Figure 4. Rat Z-6's mean cumulative reinforcements over 1-hr sessions as a function of ethanol concentration. Each point is a mean based on observations from 10 sessions, except for those points at 0%, which are means from 40 sessions. Open circles represent values from sessions following the series of sessions at 32%.

that the low rate of responding on the side opposite ethanol was due to the fact that early in the experiment such responses resulted in presentations of an empty dipper cup, and thus, such responding may have been extinguished. Two considerations suggest that this interpretation is not correct. First, in experiments where water replaced ethanol solutions, responding was not maintained (Meisch and Thompson, 1972, 1973, 1974a, 1974b).

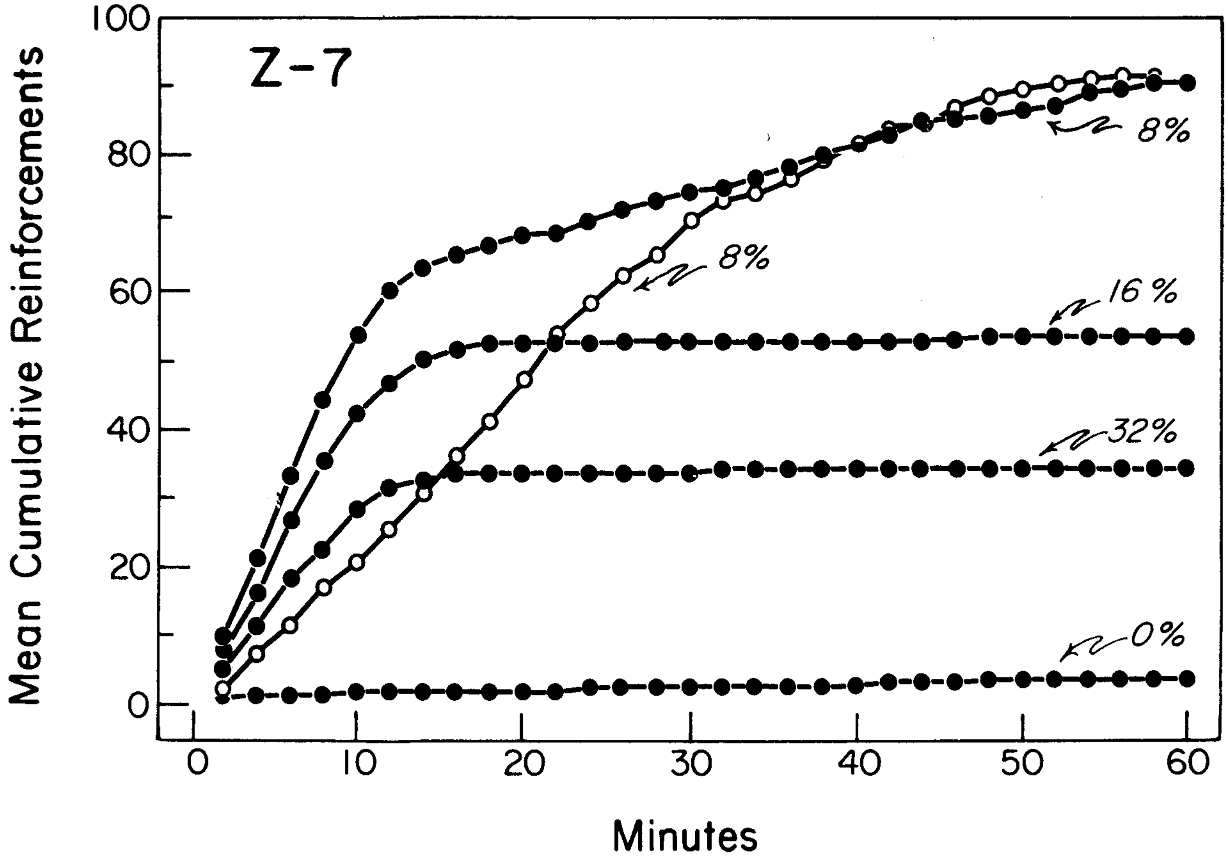

Figure 5. Rat Z-7's mean cumulative reinforcements over 1-hr sessions as a function of ethanol concentration. Each point is a mean based on observations from 10 sessions, except for those points at 0%, which are means from 40 sessions. Open circles represent values from sessions following the series of sessions at 32%.

Second, the rats had more than 40 sessions with water on the side opposite ethanol, and this should have provided sufficient opportunity to make contact with its presence.

The present procedure of providing concurrent access to water and ethanol has several advantages. It eliminates the necessity for separate water control sessions. Certain variables, such as drugs, may be efficiently

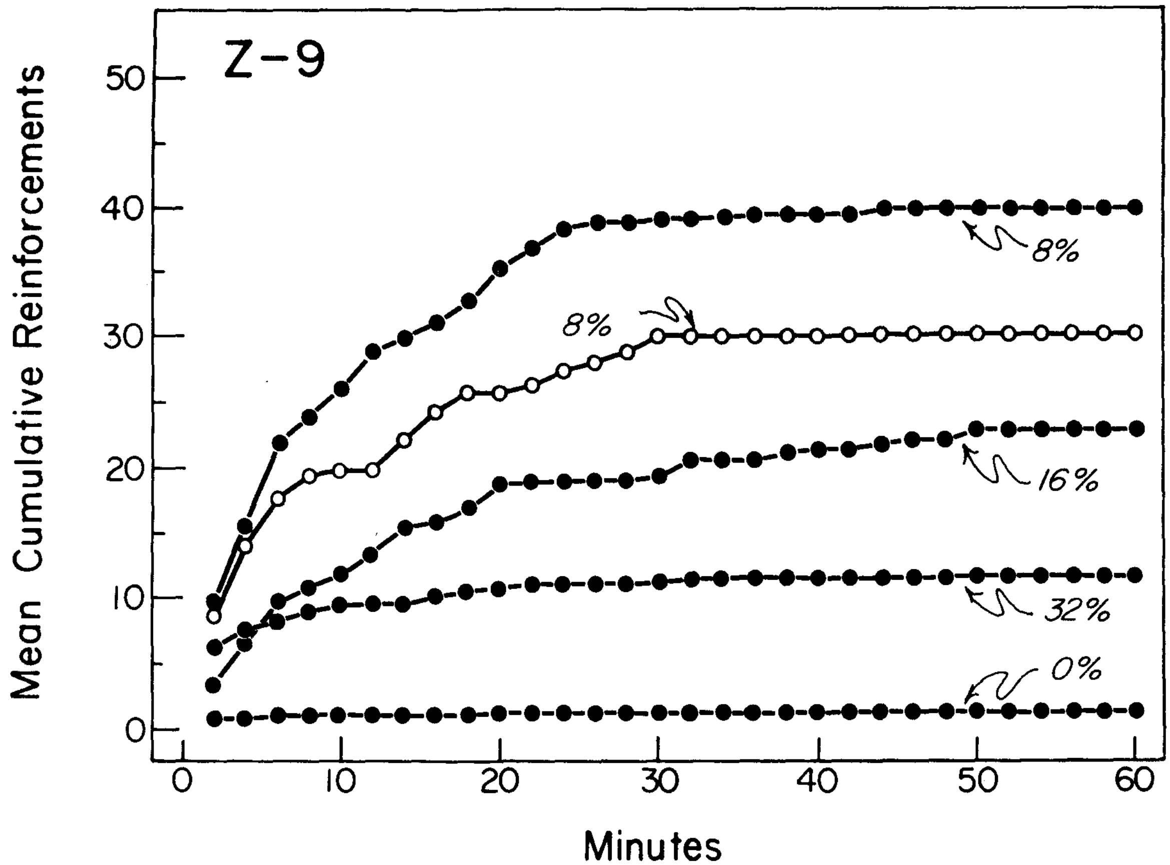

Figure 6. Rat Z-9's mean cumulative reinforcements over 1-hr sessions as a function of ethanol concentration. Each point is a mean based on observations from 10 sessions, except for those points at 0%, which are means from 40 sessions. Open circles represent values from sessions following the series of sessions at 32%.

studied, for the effects of the variables on water and ethanol intake may be examined within the same session. The concurrent procedure also controls within sessions for factors affecting liquid intake that may fluctuate across days. The procedure also provides the additional dependent variable of choice of liquid location. In future experiments the procedure may be used to examine choice between ethanol solutions and other liquids. Finally, this

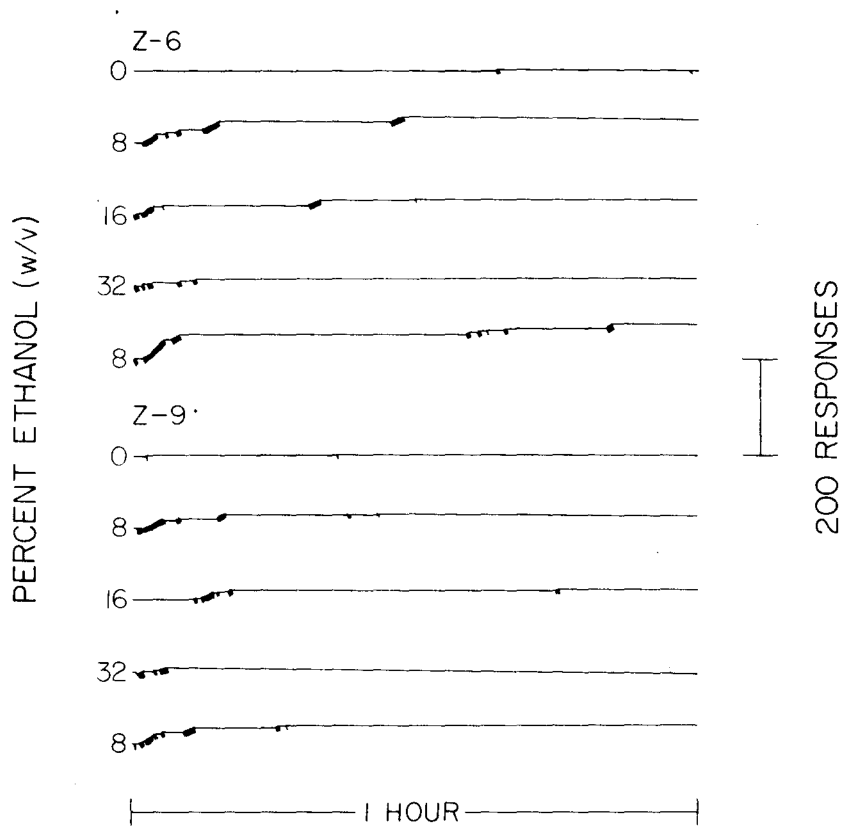

Figure 7. Representative cumulative records for rats Z-6 and Z-9 at each concentration. Numbers at the left designate the ethanol concentration. Time is indicated along the abscissa, and responses are cumulated along the ordinate. Slash marks indicate dipper presentations. Each record for each rat was selected on the basis of being closest to the mean value at a particular concentration.

procedure should facilitate comparison of results with those from other laboratories, since most investigators have employed a concurrent water--ethanol option.

The results of this experiment differ from those of Myers and Carey's study (1961). They found that water reinforcements increased as ethanol concentration increased. This discrepancy in results between the two studies is most likely a reflection of the various procedural differences.

The present results extend the generality of conditions under which ethanol serves as a reinforcer to conditions where water is concurrently available. These results also replicate certain earlier findings: (1) above 8%, reinforcements decreased with increases in the ethanol concentration; (2) quantity (mg) consumed increased with increases in the concentration; and (3) the highest rate of intake occurred at the beginning of the session (Meisch and Thompson, 1972, 1974a, 1974b). This time course of intake is that which should results in ethanol's producing the greatest behavioral effects (Meisch and Thompson, 1974a).

REFERENCES

Meisch, R.A. and Thompson, T. Ethanol reinforcement: Effects of concentration during food deprivation. In O. Forsander and K. Eriksson (Eds.), Biological aspects of alcohol consumption. Helsinki: Finnish Foundation for Alcohol Studies, 1972.

Meisch, R.A. and Thompson, T. Ethanol as a reinforcer: Effects of fixed-ratio size and food deprivation. Psychopharmacologia, 1973, 28, 171-183.

Meisch, R.A. and Thompson, T. Ethanol intake as a function of concentration during food deprivation and satiation. Pharmacology Biochemistry and Behavior, 1974, 2, in press (a).

Meisch, R.A. and Thompson, T. Rapid establishment of ethanol as a reinforcer for rats. Psychopharmacologia, 1974, 37, 311-321 (b).

Myers, R.D. Effects of meprobamate of alcohol preference and on the stress of response extinction in rats. Psychological Reports, 1961, 8, 385-391 (a).

Myers, R.D. Changes in learning, extinction, and fluid preferences as a function of chronic alcohol consumption in rats. Journal of Comparative and Physiological Psychology, 1961, 54, 510-516, (b).

Myers, R.D. and Carey, R. Preference factors in experimental alcoholism. Science, 1961, 134, 469-470.

Myers, R.D. and Veale, W.L. The determinants of alcohol preference in animals. In B. Kissin and H. Begleiter (Eds.), The biology of alcoholism, Vol. 1. New York: Plenum Press, 1971.

Wallgren, H. and Barry, III, H. Actions of Alcohol, Vol. 2. Chronic and clinical aspects. New York: Elsevier, 1970.

APPENDIX

Table 1

Significance levels (p) and t Values of the Differences in the Number of Liquid Reinforcements Obtained on the Left and Right Sides

Ethanol Concentration W/V Left	vs Right	Rat Z-9 p	Rat Z-9 t	Rat Z-7 p	Rat Z-7 t	Rat Z-6 p	Rat Z-6 t	Rat Z-2 p	Rat Z-2 t
E	E	NS	0.46	NS	1.94	NS	1.45	.01**	4.86
8	8	NS	1.39	NS	1.08	NS	0.12	NS	0.12
0	0	NS	0.85	.05**	2.66	.05**	2.89	.001**	12.83
8	8	NS	0.00	NS	1.44	NS	0.29	NS	1.13
0	0	NS	1.90	NS	1.54	NS	1.63	.01**	3.92
16	16	NS	0.20	NS	1.26	NS	0.25	NS	0.52
0	0	NS	0.43	NS	1.46	NS	1.71	NS	1.43
32	32	NS	1.34	NS	1.57	NS	0.07	NS	0.33
0	0	.05**	2.43	NS	0.50	.05**	2.32	.05**	2.74
8	8	NS	1.55	NS	1.13	NS	0.36	NS	0.29

* left greater than right

** right greater than left

E = Empty dipper (no liquid present in reservoir).

Table 2

Rat Z-2's reinforcements obtained and volume consumed as a function of ethanol concentration and location (left side versus right side)

Ethanol Concentration W/V	Reinforcements		Volume Consumed (ml)	
	Left $\bar{x} \pm$ (S.E.)	Right $\bar{x} \pm$ (S.E.)	Left $\bar{x}$	Right $\bar{x}$
E	2.20* (0.97)	13.00 (2.00)	---	---
8	41.40 (0.98)	41.80 (3.32)	4.97	5.02
0	0.20 (0.20)	5.00 (0.32)	0.02	0.60
8	37.20 (1.66)	41.80 (3.72)	4.46	5.02
0	0.00 (0.00)	3.80 (0.97)	0.00	0.46
16	35.40 (1.12)	34.40 (1.57)	4.25	4.13
0	1.00 (0.77)	2.40 (0.60)	0.12	0.29
32	19.80 (1.16)	19.00 (2.14)	2.38	2.28
0	0.60 (0.40)	3.20 (0.86)	0.07	0.38
8	53.80 (3.07)	52.60 (2.82)	6.46	6.31

* n = 5
E = Empty dipper (no liquid present in reservoir).

Table 3

Rat Z-6's reinforcements obtained and volume consumed as a function of ethanol concentration and location (left side versus right side)

Ethanol Concentration W/V	Reinforcements				Volume Consumed (ml)	
	Left $\bar{x} \pm$ (S.E.)		Right $\bar{x} \pm$ (S.E.)		Left $\bar{x}$	Right $\bar{x}$
E	1.60*	(0.93)	4.40	(1.08)	---	---
8	44.00	(4.84)	43.20	(4.83)	5.28	5.18
0	0.20	(0.20)	3.00	(0.95)	0.02	0.36
8	57.40	(4.35)	58.80	(2.13)	6.89	7.06
0	0.20	(0.20)	1.00	(0.45)	0.02	0.12
16	30.00	(2.28)	30.80	(2.22)	3.60	3.70
0	0.60	(0.24)	1.40	(0.40)	0.06	0.17
32	16.40	(2.66)	16.60	(1.29)	1.97	1.99
0	0.20	(0.20)	2.40	(0.93)	0.02	0.29
8	55.60	(5.11)	57.60	(2.25)	6.67	6.91

* n = 5

E = Empty dipper (no liquid present in reservoir).

Table 4

Rat Z-7's reinforcements obtained and volume consumed as a function of ethanol concentration and location (left side versus right side)

Ethanol Concentration W/V	Reinforcements		Volume Consumed (ml)	
	Left $\bar{x}$ ± (S.E.)	Right $\bar{x}$ ± (S.E.)	Left $\bar{x}$	Right $\bar{x}$
E	3.20* (0.86)	12.80 (4.87)	---	---
8	72.40 (3.23)	67.80 (2.80)	8.69	8.14
0	3.20 (0.73)	9.20 (2.13)	0.38	1.10
8	95.20 (3.48)	86.80 (4.66)	11.42	10.42
0	3.20 (0.37)	2.20 (0.37)	0.38	0.26
16	54.40 (3.34)	49.40 (1.83)	6.53	5.93
0	1.80 (0.49)	2.60 (0.24)	0.22	0.31
32	31.20 (3.15)	36.60 (1.36)	3.74	4.39
0	2.40 (1.50)	3.20 (0.58)	0.29	0.38
8	97.40 (4.65)	89.60 (5.10)	11.69	10.75

* n = 5

E = Empty dipper (no liquid present in reservoir).

Table 5

Rat Z-9's reinforcements obtained and volume consumed as a function of ethanol concentration and location (left side versus right side)

Ethanol Concentration W/V	Reinforcements				Volume Consumed (ml)	
	Left $\bar{x}$ ± (S.E.)		Right $\bar{x}$ ± (S.E.)		Left $\bar{x}$	Right $\bar{x}$
E	1.00*	(0.55)	1.40	(0.68)	---	---
8	37.80	(5.05)	29.00	(3.85)	4.54	3.48
0	2.40	(0.51)	1.80	(0.49)	0.29	0.22
8	41.00	(3.08)	41.00	(6.24)	4.92	4.92
0	0.40	(0.24)	2.80	(1.24)	0.05	0.34
16	23.40	(2.09)	22.80	(2.20)	2.81	2.74
0	0.40	(0.24)	0.60	(0.40)	0.05	0.07
32	13.00	(1.41)	10.40	(1.33)	1.56	1.25
0	0.80	(0.37)	2.80	(0.73)	0.10	0.34
8	27.40	(1.66)	33.80	(3.77)	3.29	4.06

E= Empty dipper (no liquid present in reservoir).

* n = 5