Pharmacology Biochemistry & Behavior, Vol. 14, pp. 871–876, 1981. Printed in the U.S.A.

A Precision Drinking Device for Rats Tested with Water, Etonitazene, and Ethanol[1]

PATRICK M. BEARDSLEY[2] AND RICHARD A. MEISCH[3]

Psychiatry Research Unit, University of Minnesota, Minneapolis, MN 55455

Received 21 October 1980

BEARDSLEY, P. M. AND R. A. MEISCH. *A precision drinking device for rats tested with water, etonitazene, and ethanol.* PHARMAC. BIOCHEM. BEHAV. **14**(6) 871–876, 1981.—An inexpensive system for detecting tongue licking and for delivering liquids to rats is described. Liquids were dispensed under a heterogenous chain schedule of reinforcement in which depressions of a lever were required before tongue licking produced liquid delivery. Three rats drank water dispensed by the system at lowest, highest, and midmost valve settings. The volume of water delivered increased logarithmically from 0.00270 ml to 0.01180 ml across valve settings. In additional tests, drug-experienced rats were tested with either 5.0 μg/ml etonitazene HCl, water, or 8% w/v ethanol available with the valve at its widest setting. More of the drug solutions were consumed than water suggesting that the drugs served as reinforcers. Intakes of both drugs were compared with intakes previously determined in dipper delivery systems using the same rats. This liquid delivery system has several advantageous features in that it is inexpensive to construct, allows quick and easy control of the volume delivered, minimizes evaporation, and, along with the heterogenous chain schedule of reinforcement, insures that liquid is delivered only when oral contact is made with it.

Drinking device Heterogenous chain schedule of reinforcement Etonitazene Ethanol Rats

COMMON methods of delivering liquids to rats include the presentation of filled dipper cups and the delivery of droplets through drinking spouts controlled by electronic drinkometers. Delivering liquids by refilling dipper cups has certain disadvantages. The amount delivered can only be varied by exchanging different sizes of dipper cups. Machining a dipper cup for each of many volumes is laborious and expensive. Secondly, a reservoir is needed which, because it must be partially opened for dipper refilling, allows evaporation. Thirdly, substantial evaporation can occur from the dipper cup itself, which could lead to imprecise measurements of volume consumed. Finally, and most importantly, there is no assurance that the animal contacts and drinks liquid upon each dipper presentation. This can be particularly problematic when an animal is under a drug's influence. Electronic drinkometer-equipped systems have been designed which eliminate most of the problems associated with dipper cup delivery systems (e.g., [1, 2, 3]). However, when previous drinkometer-equipped systems have gained precision and offered ease in controlling a range of small volumes (less than 0.05 ml), they have done so at considerable expense, for instance, by employing costly syringe pumps (e.g., [1]).

The present drinking system was designed to eliminate the problems associated with dipper cup delivery systems and to precisely deliver a range of small volumes of liquids at an inexpensive construction cost. Its use was tested under conditions in which three different volumes of water, and the drugs etonitazene HCl and ethanol, were delivered following completion of a heterogenous chain schedule of reinforcement [4].

METHOD

Subjects

Three adult, experimentally naive, male, Long-Evans descent hooded rats (Blue-Spruce Farms), approximately 2 years old at the beginning of the study, were tested during daily 1 hr experimental sessions.

Apparatus

The drinking device is shown in Fig. 1. A vertically supported Becton-Dickinson H575-G 35 cc glass syringe serves as a reservoir. Weight on the syringe plunger is supplied by a 61.26 g, hollowed, Plexiglas cylinder, $7^{3}/_{16}$ in. (18.26 cm) long with a $1^{7}/_{32}$ in. (3.10 cm) diameter. The syringe is fitted with a screw-type Luer-Lok stopcock (Scientific Products #3154) to enable removal without spillage. Polypropylene

[1]This research was supported by NIAAA grant AA 00299 and by NIDA grant DA 00944. We thank Gregory A. Lemaire and James P. Goldberg for their comments on an earlier version of this manuscript.

[2]NIDA Predoctoral Fellowship Awardee, DA 05111. Send reprint requests to Patrick M. Beardsley, Psychiatry Research Unit, Box 392 Mayo, University of Minnesota, Minneapolis, MN 55455.

[3]Research Scientist Development Awardee, DA 00007.

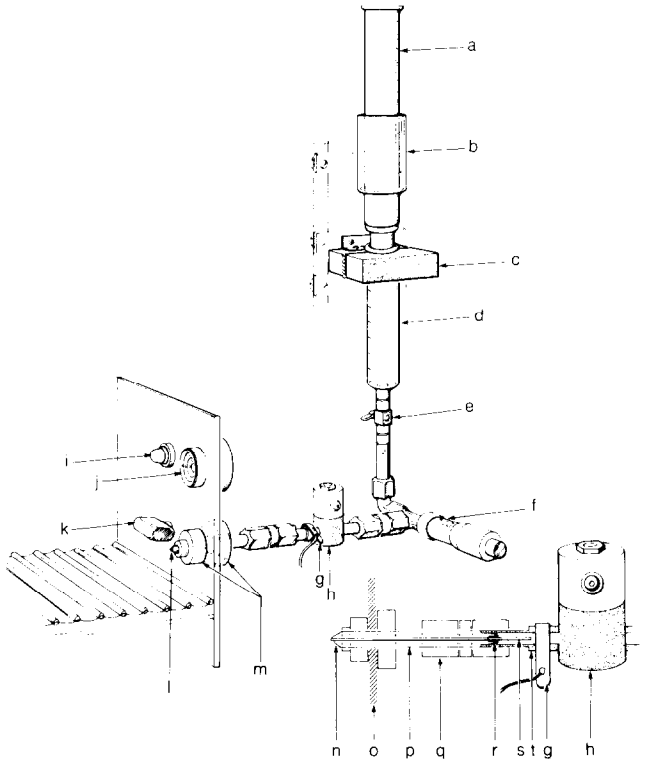

FIG. 1. Drinking device and internal construction from the solenoid to the drinking spout (inset). (a) Plexiglas cylinder; (b) cylinder guide; (c) syringe support stand; (d) 35 cc syringe; (e) stopcock; (f) micrometric capillary valve; (g) contact clip; (h) liquid solenoid valve; (i) white-jewelled lamp; (j) Sonalert; (k) response lever; (l) spout; (m) plastic sleeve; (n) bevelled spout tip; (o) intelligence panel; (p) glass rod; (q) tube-to-tube connector; (r) copper clip; (s) stainless steel tube; (t) hose barb.

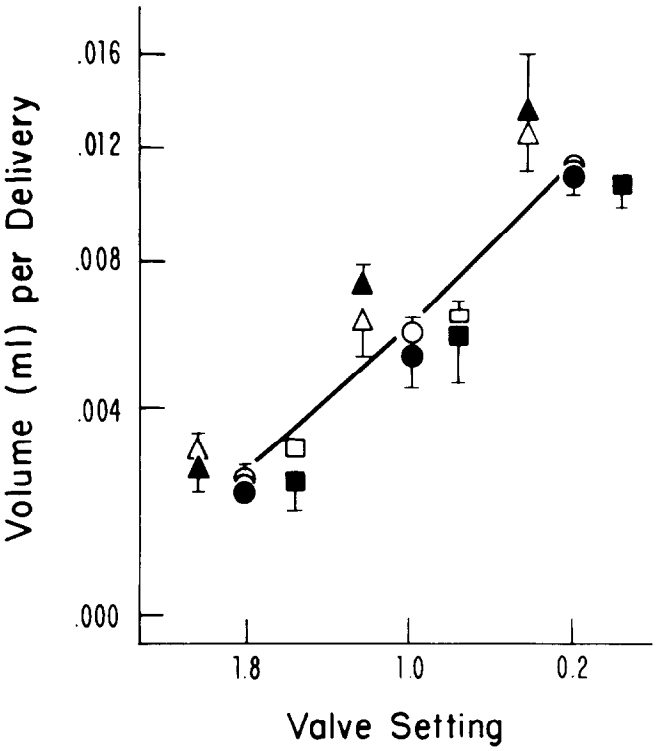

FIG. 2. Mean ml of water per delivery for each rat at each valve setting for the test and retest conditions. Filled symbols: test conditions. Unfilled symbols: retest conditions. Triangles, circles, squares: rats L-5, L-8, and L-10, respectively. Each symbol represents the mean of 5 consecutive session values at a particular valve setting; each session value being a mean of the volume of water delivered per delivery during that session. Brackets indicate the range of the means used to compute each point. Where no brackets appear, the range of the means fell within the enclosed symbol. The line passing through the symbols connects the group means (the means of each of 3 rats × 2 conditions, i.e., test and retest conditions, each). Note that decreases in the valve setting result in increases in the valve opening, (i.e., at valve setting 0.0, valve is completely open; at valve setting 2.0, valve is completely closed).

tubing, $^{5}/_{16}$ in. (7.94 mm) o.d./0.035 in. (0.889 mm) wall, and an elbow tube-to-tube connector (Cole-Parmer #6383-20) connects the syringe to the intake side of a micrometric capillary valve (Cole-Parmer #3235). The output side of the micrometric capillary valve is connected to the intake port of a liquid solenoid valve (Gould #21381-24VDC). The ports of the liquid solenoid valve are fitted with brass hose barbs, $^{1}/_{8}$ in. (3.18 mm) i.d. Polypropylene tubing is pressed over both hose barbs. The tubing is then extended into union, tube-to-tube connectors (Cole-Parmer #6381-20) connecting the output side of the micrometric capillary valve to the input port of the liquid solenoid valve, and, on the other side, connecting a $3^{9}/_{16}$ in. (9.05 cm) long, hollowed, glass rod, $^{5}/_{16}$ in. (8 mm) o.d./0.07 in. (1.75 mm) i.d., to the output port. The glass rod extends $1^{3}/_{32}$ in. (2.78 cm) into the operant chamber and is secured to the intelligence panel and supported by a $^{27}/_{32}$ in. (2.14 cm) plastic sleeve. The tip of the glass rod located in the operant chamber is smoothed and tapered.

A $4^{7}/_{16}$ in. (11.27 cm) long, stainless steel tube, 0.0655 in. (1.66 mm) o.d./0.0415 in. (1.05 mm) i.d., runs through the glass rod and into a copper clip which is press-fitted into the inside walls of the brass hose barb at the output port of the liquid solenoid valve. This clip, item "r" in the insert of Fig. 1, was formed by bending a copper strip into the shape of the letter "W" and boring a hole at its midpoint through which the stainless steel tube is passed. The copper clip serves as a bridge allowing electrical continuity between the stainless steel tube and the brass hose barb. The stainless steel tube is cemented by epoxy to the inside walls of the glass rod, and is flush with the exposed tip of the rod. The lower bevel of the glass rod's tip is cut out $^{1}/_{16}$ in. (1.59 mm) vertically by $^{1}/_{32}$ in. (0.79 mm) horizontally. A $^{1}/_{16}$ in. (1.59 mm) wide by $^{3}/_{16}$ in. (4.76 mm) long copper strip is passed through the glass rod's lower bevel, under, and press fitted with the stainless steel tubing until one end is flush with the exposed end of the stainless steel tubing. This copper strip was included to maximize contact area sensitivity; however, in tests subsequent to the present report the exclusion of this strip had no observable effect on sensitivity nor on drinking behavior. Electrical continuity thus extends from the copper strip at the lower bevel of the glass rod, through the stainless steel tubing, into the copper clip and through the brass hose barb at the output port of the liquid solenoid valve (see Fig. 1, insert). A wire, clipped to the brass hose barb at the output port of the liquid solenoid valve, serves as the input line to a

Coulbourn Instruments S26-01 Contact Input module. The contact input module employs a maximum 1 μA sense current and has a resistance threshold of 2 MΩ. When programmed, tongue contacts of the exposed copper strip at the glass rod's lower bevel result in a 40 msec delivery of liquid with a latency of 6 to 10 msec following tongue contact. Costs for the micrometric capillary valve, syringe-reservoir, liquid solenoid valve, stopcock valve, and all tubing connectors total less than $85.00. Additional costs for the glass and stainless steel rods and raw materials for the syringe holder and syringe weight brought construction costs (less operant chamber and programming equipment) to between $100 and $125.

The operant chamber itself is 11 in. (27.94 cm) long by 7 1/2 in. (19.05 cm) wide by 6 in. (15.24 cm) in height. On the left side of the intelligence panel, a lever is mounted 1 1/2 in. (3.81 cm) above the floor, and 2 3/4 in. (6.99 cm) directly above the lever a 1.12 W white-jewelled lamp is positioned. The drinking spout (the tip of the glass rod) emerges 1 1/2 in. (3.81 cm) above the floor, 1 7/8 in. (4.76 cm) to the right of the lever. The speaker of a Sonalert (Mallory #S6628-24VDC) is positioned 2 3/4 in. (6.99 cm) directly above the drinking spout. A 4.76 W house light is centered on top of the operant chamber's Plexiglas cover 3 in. (7.62 cm) from the chamber's back panel. The operant chamber and drinking device are enclosed in a ventilated, sound-attenuating, plastic ice chest.

Procedure

The rats were allowed access to water during experimental sessions and for a 30 min period following each experimental session in their home cages. During sessions, the white house light was continually lit and water deliveries were available on a heterogenous chain Fixed Ratio 1 (lever press) Fixed Ratio 1 (20) (spout contact) schedule of reinforcement; a single lever press allowed 20 reinforced spout contacts, i.e., chain FR 1 FR 1 (20). The onset of the white-jewelled lamp signalled the first component of the chain, in which one lever press was necessary to initiate the second component. The offset of the white-jewelled lamp and the sounding of the Sonalert signalled the second component of the chain, during which spout contacts resulted in water deliveries. Following the 20th reinforced spout contact the Sonalert extinguished and the white-jewelled lamp was again lit. Neither spout contacts during the first component nor lever presses during the second component had programmed consequences. This particular schedule was employed because it required a typically used, quantifiable instrumental response (lever press) to be emitted prior to gaining access to liquid. Although a lick response could have been used alone as the required instrumental response, lick responding is often atypical of other instrumental behavior [2] and bears respondent-like properties [7,8]. Use alone of the licking response without a lever press requirement would have thus made the data less comparable with other research employing instrumental response requirements.

Volume of liquid consumed was determined by changes in weight of the syringe reservoir. That is, prior to each session, the syringe reservoir, filled with liquid, was weighed on a Mettler Platform Balance (PL 1200) and then reweighed immediately after each session. The change in weight, converted into a volume measure based on the relative density of water at laboratory temperatures, was then used as the measure of volume consumed.

Following acquisition of lever press and spout contact responses, settings on the micrometric capillary valve of 1.8, 1.0 and 0.2 were tested with each rat in the following order: 1.8, 1.0, 0.2, 1.0, 1.8, 0.2. Value settings of 0.2, 1.0, and 1.8 were chosen for testing since they included the practical limits of the valve's control (settings of 0 and 2.0 completely opened and closed the valve) while being spaced maximally apart from one another. Decreases in the valve setting result in increases of the valve opening. During the retest condition at valve setting 0.2, rat L-10 sickened and died; consequently, its data at this condition were excluded from the following report. Changes in one valve setting to the next were made following 5 consecutive sessions in which there

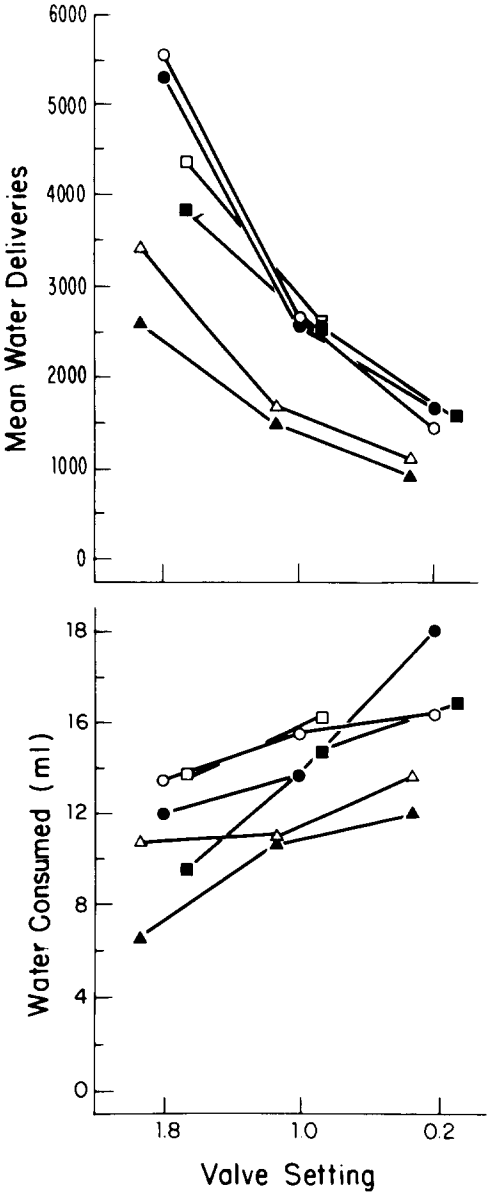

FIG. 3. Mean deliveries (upper panel) and volume consumed (lower panel) obtained by each rat at each valve setting for the test and retest conditions. Filled symbols: test condition. Unfilled symbols: retest condition. Triangles, circles, squares: rats L-5, L-8, and L-10, respectively. Each symbol represents the mean of 5 consecutive session values.

TABLE 1

MEAN NUMBER (N=5) OF DELIVERIES, VOLUME CONSUMED, AND VOLUME PER DELIVERY OF 5.0 μg/ml ETONITAZENE (Etz.), 8% w/v ETHANOL, OR WATER, AND MEAN DRUG INTAKE

Rat	Liquid	Mean Deliveries	Mean ml Consumed	Mean ml/Delivery	Mean μg/kg Body Weight/hr
	5.0 μg/ml Etz. (test)	355.0	5.68	0.0161	31.8
C-10	0.0 μg/ml Etz. (water)	54.2	1.15	0.018*	—
	5.0 μg/ml Etz. (retest)	314.2	4.81	0.0152	27.0
	5.0 μg/ml Etz. (test)	396.0	7.31	0.0185	60.7
B-3	0.0 μg/ml Etz. (water)	56.8	0.90	0.017	—
	5.0 μg/ml Etz. (retest)	451.4	6.99	0.0158	58.1
	8% w/v ethanol (test)	308	4.31	0.0142	0.314†
T-2	0% w/v ethanol (water)	56	1.23	0.024‡	—
	8% w/v ethanol (retest)	765	8.76	0.0119	0.638†

*N=4; i.e., 1 session had 0 deliveries and 0 volume consumed.
†Mean g/kg body weight/hr.
‡See text.

were no systematic increasing or decreasing trends in the number of liquid deliveries.

RESULTS

Figure 2 shows the mean milliliters of water per delivery for each rat at each valve setting for the test and retest conditions. At each individual valve setting, similar volumes of water were delivered for each rat during both the test and retest conditions. The mean milliliters of water per delivery increased from 0.00270 at valve setting 1.8 to 0.00615 at valve setting 1.0 to 0.01180 at valve setting 0.2. As can be seen from Fig. 2 there was a logarithmic increase in the volume delivered with decrements in the valve setting. The range of each group mean was small, and in no case did the range at a valve setting overlap with ranges of any other valve setting.

Figure 3 (upper panel) shows that mean liquid deliveries for each rat at each valve setting for the test and retest conditions decreased with increases in the valve opening. Although there were sizeable differences in the number of liquid deliveries among the rats at valve setting 1.8, these differences decreased at lower valve settings.

Figure 3 (lower panel) shows that with decrements in the valve setting there were increments in the volume of water drunk per session. This was true for each rat during both the test and retest conditions. In 7 of 8 possible comparisons, more water was drunk during the retest than the test condition at each valve setting (again, note there was no completed retest condition for rat L-10 at valve setting 0.2). Combining test and retest conditions (N=30; 3 rats × 2 conditions × 5 sessions each) group mean water consumption increased from 10.99 ml, to 13.63 ml, to 15.38 ml per session with respective decrements in the valve setting from 1.8 to 1.0 to 0.2.

ADDITIONAL TESTS USING DRUG SOLUTIONS

Additional tests were conducted using the device to deliver solutions of etonitazene HCl or ethanol. Rats C-10 and B-3 were allowed access to 5.0 μg/ml etonitazene, water, and 5.0 μg/ml etonitazene (retest) and rat T-2 to 8% w/v ethanol, water, and 8% w/v ethanol (retest) in those orders. The particular drug made available to each rat had previously served as a reinforcer in standard dipper delivery experimental chambers (Beardsley, Lemaire and Meisch, unpublished data).

The schedule on which liquid was available differed slightly for each rat. Rat C-10 was allowed access to liquids on a heterogenous chain Fixed Ratio 4 (lever press) Fixed Ratio 1 (20) (spout contact) Limited Hold 10 sec schedule of reinforcement in which following 4 lever presses, spout contacts were reinforced for 10 sec or for 20 deliveries, whichever came first, i.e., chain FR 4 FR 1 (20) LH 10 sec. Rat B-3 was allowed liquid access on a chain FR 8 FR 1 (20) LH 30 sec schedule and rat T-2 was given access on a chain FR 16 FR 1 (20) schedule.

For all rats stimulus conditions during each component of the chain schedule of reinforcement were identical to those described previously except the white-jewelled lamp flickered at 10 times per sec during the first component when drug solutions were available. Daily experimental session lengths were 2 hr in duration for rats C-10 and B-3, and 3 hr in duration for rat T-2. The rats had unlimited access to water between sessions in their home cages. The micrometric capillary valve was at setting 0.2 throughout all tests.

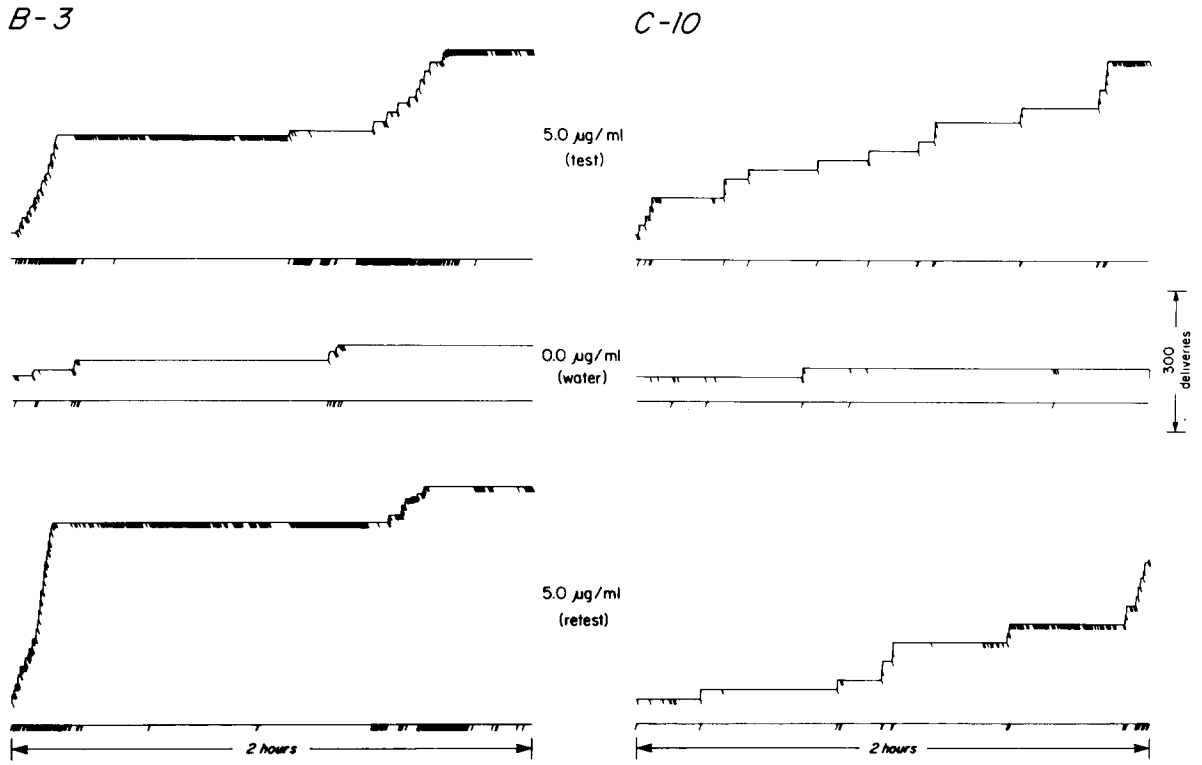

FIG. 4. Cumulative records of the performance of rats B-3 and C-10 during the 5.0 µg/ml etonitazene test and retest conditions and during the water condition. Each record was selected on the basis of representing the number of liquid deliveries closest to the mean for its respective condition. Event pen pips represent lever presses. Increments in the stepping pen indicate reinforced spout contacts. Stepping pen pips indicate unreinforced spout contacts.

RESULTS

Each rat obtained more drug deliveries and similarly drank more of the available drug solution than water (Table 1). The greater drug than vehicle (water) drinking indicates that the available drug solutions served as a reinforcer for each rat [5,6].

Table 1 shows the mean volume of liquid delivered per delivery during all conditions. With the exception of when water was available for T-2, the mean volume per delivery did not vary more than ±0.006 ml across all conditions. The high volume per delivery determination obtained when water was present (0.24 ml per delivery) for T-2 was, in part, accounted for by one anomalous session value in which the individually calculated volume per delivery of 0.035 ml was obtained. In general, the volume dispensed per delivery did not vary systematically either as a function of type of liquid available or number of liquid deliveries obtained.

Figure 4 presents sample cumulative records for rats C-10 and B-3 from each condition. When water was the available liquid there was little drinking; few unreinforced spout contacts (first component contacts), or nonessential lever presses (i.e., lever presses not initiating the second component of the chain schedule) occurred. However, with 5.0 µg/ml etonitazene a different pattern emerged for both rats. There was much more drinking. Also, many more unreinforced spout contacts occurred. The increase in unreinforced spout contacts was especially pronounced with B-3; bouts of hundreds of unreinforced spout contacts occurred after an initial bout of drinking. These unreinforced spout contacts were followed by bouts of nonessential lever presses before etonitazene drinking resumed. Additionally, a greater number of available liquid deliveries were not obtained when etonitazene was present. That is, the second component of the chain schedule was more frequently terminated by the expiration of the limited hold when 5.0 µg/ml etonitazene was present. This was true for both rats, but again was more pronounced with rat B-3 which missed, on the average per session, 913.9 available etonitazene deliveries and only 43.2 available water deliveries.

Intakes of etonitazene in the present study (see Table 1) were similar to previous etonitazene intakes obtained with rats C-10 and B-3 at an identical etonitazene concentration and equal session duration when a dipper delivery system was used (Beardsley and Meisch, unpublished data). Rat C-10 averaged 31.7 µg/kg body weight/hr in the dipper delivery system and 29.4 µg/kg body weight/hr in the present system (mean of test and retest values, i.e., N=10; 2 conditions × 5 sessions each). Rat B-3 averaged 76.1 µg/kg body weight/hr in the dipper delivery system and 59.4 µg/kg body weight/hr in the present system. These similarities in intake persisted despite differences in schedule of availability, age of rats, and experimental chamber used.

Intake of ethanol for rat T-2 in the present study was dissimilar to previous intake determined using a dipper delivery system (Beardsley, Lemaire and Meisch, in preparation). When tested in the dipper delivery system during ses-

sions of identical duration and ethanol concentration as used in the present study, rat T-2's ethanol intake was 0.751 g/kg body weight/hr. In the present study, rat T-2's ethanol intake was 0.476 g/kg body weight/hr (mean of test and retest values, i.e., N=10; 2 conditions × 5 sessions each). However, it should be noted that ethanol intake during the retest condition of the present study was 0.638 g/kg body weight/hr which was over twice that of the test condition (0.314 g/kg body weight/hr). Reasons for the difference in results between the test and retest conditions are unknown.

GENERAL DISCUSSION

The present drinking system provided several advantages over dipper delivery systems and did so at a relatively inexpensive cost. First, it allowed quick and easy control of the volume delivered between 0.00270 and 0.01180 ml. Presumably the range of liquid delivered could be further expanded by manipulating the duration of delivery from that used in the present study (40 msec) or by adding weight to the hollowed, Plexiglas cylinder used to depress the syringe plunger.

Secondly, evaporation was minimized by the use of a closed reservoir. Utilization of completely closed reservoirs in dipper delivery systems are precluded by the necessity of dipper cup refilling operations.

Thirdly, and most importantly, the drinking device, in conjunction with the heterogenous chain schedule of reinforcement, insured that liquid was delivered only when oral contact was made with it. The assurance of oral contact with dispensed liquid is particularly important in research involving the oral self-administration of drugs. For instance, in previous research (Beardsley and Meisch, unpublished data) it was observed that rats, orally self-administering 5.0 μg/ml etonitazene, would complete fixed-ratio lever press requirements repeatedly for dipper presentations of the drug yet would not drink the presented liquid. This repeated occurrence of not drinking available drug followed periods in which some etonitazene had been drunk which suggests the influence of a drug effect. The present drinking device, as programmed, prevented dispensing of liquids unless there was tongue contact on the drinking spout, and it enabled recording of tongue contacts when liquid was not available. Also, when a limited hold was imposed on the second component of the chain schedule as with rats C-10 and B-3, the system allowed recording of missed available liquid deliveries.

REFERENCES

1. Hulse, S. H. A precision liquid feeding system controlled by licking behavior. *J. exp. Analysis Behav.* **3**: 1–3, 1960.
2. Justesen, D. R., D. M. Levinson and M. Daley. A modification of the Hulse lickerandum: Preliminary studies and suggested applications. *Psychon. Sci.* **7**: 111–112, 1967.
3. Lal, H. and J. P. Zabik. Operant ingestion (oral) of ethanol: Effects of behavioral contingencies, disulfiram pretreatment and morphine dependence. In: *Drug Addiction: Experimental Pharmacology, Vol. 1*, edited by J. M. Singh and H. Lal. New York: Futura Publishing Co., 1972, pp. 61–85.
4. Kelleher, R. T. and L. R. Gollub. A review of positive conditioned reinforcement. *J. exp. Analysis Behav.* **5**: 543–597, 1962.
5. Meisch, R. A. and L. J. Stark. Establishment of etonitazene as a reinforcer for rats by use of schedule-induced drinking. *Pharmac. Biochem. Behav.* **7**: 195–203, 1977.
6. Meisch, R. A. and T. Thompson. Rapid establishment of ethanol as a reinforcer for rats. *Psychopharmacologia* **37**: 311–321, 1974.
7. Miller, N. A. and R. C. De Bold. Classically conditioned tongue-licking and operant bar pressing recorded simultaneously in the rat. *J. comp. physiol. Psychol.* **59**: 109–111, 1965.
8. Patten, R. L. and E. B. Deaux. Classical conditioning and the licking response in rats. *Psychon. Sci.* **4**: 21–22, 1966.