(*11*). A balloon was implanted in each subject intraperitoneally through a midventral incision, and the end of the balloon was drawn through the cloaca to the outside. The balloons of half the group were slightly inflated; all females were tested 16 to 18 hours later. Before implant the groups were similar, but inhibition of the release call occurred only in the distended females (Table 1). The release call returned within minutes of deflation.

The above experiments suggest that in *R. pipiens* one mechanism for pituitary regulation of breeding behavior involves the ability of [Arg^8]vasotocin to cause water accumulation and subsequent internal pressure. This is the first time this relationship has been described, and more work is necessary to test its generality (*12*). This mechanism has ecological relevance, since water is obligatory for amphibian reproduction, and moisture is often the most important trigger of reproductive behavior (*13*).

CAROL DIAKOW

Biology Department,
Adelphi University,
Garden City, New York 11530

References and Notes

1. Frogs were obtained from Ward's Natural Science Establishment or from Mumley, Vermont; all were of northern origin. There is some uncertainty about the actual species provided when *Rana pipiens* are obtained from a central supply house. In order to minimize the possibility that differences between groups within an experiment are due to different species in each group, all animals in any one experiment were from the same shipment.
2. G. K. Noble and L. R. Aronson, *Bull. Am. Mus. Nat. Hist.* **80**, 127 (1942).
3. C. Diakow, *Physiol. Behav.* **19**, 607 (1977).
4. W. H. Sawyer, *Endocrinology* **75**, 981 (1964).
5. The manual stimulation method has been described (*3*), and it was shown that the results of this method parallel those obtained when male frogs are used to test the females.
6. The median latency to oviposition from the start of amplexus (clasping by males) was 2 hours 40 minutes, and ranged from 13 minutes to more than 9 hours.
7. In all experiments, frogs were housed on receipt in community tanks containing a dilute solution of Agristrep (streptomycin sulfate). When the frogs were introduced to the experiment, they were housed in pairs (first two experiments), groups (third experiment), or singly (last two experiments) in water without Agristrep. The laboratory temperature was usually 21° to 23°C, and the overhead fluorescent lights were kept on for 14 hours per day.
8. N. Mayer, *Comp. Biochem. Physiol.* **29**, 27 (1969).
9. A. Krogh, *Osmotic Regulation in Aquatic Animals* (Cambridge Univ. Press, Cambridge, 1939).
10. Resistance was measured through two electrodes 8 mm apart and 8 mm deep in solution.
11. Each frog was placed with a damp paper towel in a loosely covered jar.
12. W. Russell [*Behaviour* **7**, 113 (1954)] induced breeding behavior in a totally aquatic frog, *Xenopus laevis*, with chorionic gonadotropin.
13. S. N. Salthe and J. S. Mecham, *Physiology of the Amphibia*, B. Lofts, Ed. (Academic Press, New York, 1974), vol. 2.
14. I thank B. Colodne who assisted during the experiment with [Arg^8]vasotocin, Drs. H. Jervis and J. W. Lazar for discussion of these experiments as they were in progress, and Drs. E. Brodie, H. Grob, D. Jones, and S. Smith for comments on the manuscript.

7 November 1977; revised 18 January 1978

Sustained Release of Alcohol: Subcutaneous Silastic Implants in Mice

Abstract. *A sustained-release device for use in ethanol dependence studies in mice is described. The Silastic device, dubbed SERT (sustained ethanol release tube), holds 0.35 milliliter of 95 percent ethanol (by volume) and is implanted under the skin of the back where it releases ethanol for up to 12 hours, with no observable tissue damage. The device may be adaptable to the release of other volatile liquids or drugs, in other animals.*

Studies on the mechanisms of tolerance and dependence produced by drugs of abuse, including alcohol (ethanol), are greatly facilitated by animal models of drug intake (*1*). Many reports have been devoted to defining, discovering, and utilizing animal models of alcoholism (*2*), and the cited criteria for a useful "animal model of alcoholism" vary from author to author (*1, 3*). Although an oral route of administration would be ideal for matching the human condition of alcoholism, the view has also been taken that the route of administration is irrelevant to research examining the pharmacology of ethanol dependence and tolerance (*4*). In addition, it is impractical and tedious to administer unpalatable drugs repetitively by mouth in large samples of animals. To this end, numerous sustained-release forms of abused drugs have been reported, including morphine (*5*), pentobarbital (*6*), and amphetamine (*7*). To our knowledge, however, no sustained release forms of ethanol have been developed.

Working under the hypotheses that maintenance of continuous intoxication is the key to producing physical dependence (*4*), and that tolerance and physical dependence on ethanol can be produced by its sustained release from an implantable subcutaneous silastic tube in mice, we have developed a sustained ethanol release tube (SERT) for use in mice and other animals. The standard device is 60 mm in length and consists of Dow Corning medical grade Silastic tubing (3.35 mm inner diameter; 4.65 mm outer diameter). The SERT is sealed at one end with a siloxane polymer, closed at the other end with a glass stopper (Fig. 1), and holds 0.35 ml of liquid. The pretied neck prevents the stopper from being inserted too far and maintains the SERT in the correct position. The SERT is implanted by aseptic techniques under the skin of the back so that the sealed end is near the tail and the stoppered end protrudes through the skin at the neck of the mouse, between the ears (*8*).

We have studied the release rates of various ethanol concentrations from the SERT implants in beakers of distilled water and saline, at both room temperature and body temperature, and found that the release rates in vitro are proportional to the concentration of ethanol inside the tube (data not shown). The release rate in vivo (*9*) is faster than that in vitro, presumably in part because the ethanol that is released in the mouse is carried away by the circulation and metabolized by the liver. Although release of 95 percent (by volume) ethanol from the tube is not linear, the mean release rate in vivo from the standard SERT over 12 hours has been calculated to be 760 mg per kilogram of body weight per hour (18.2 g kg^{-1} day^{-1}), which is only slightly greater than the normal rate of metabolism of ethanol in the mouse [550 mg kg^{-1} hour^{-1} (*10*)]. We have found, furthermore, that concentrations of alcohol in the blood of implanted mice vary significantly, because of different rates of metabolism within the strain (*11*). We speculate that variable ethanol release rates from the SERT may thus occur. It is therefore desirable to screen the mice to eliminate those with very high and very low rates of alcohol metabolism (*12*). This is only for convenience, however; all animals can be used if care is taken to give each animal supplemental (intraperitoneal) injections of alcohol dependent upon its behavioral intoxication.

The rate of release of 95 percent ethanol from the SERT is ideal for maintaining blood concentrations produced by an initial intraperitoneal injection of ethanol (Fig. 2). Thus a "high" dose of 3.5 g/kg injected intraperitoneally will be maintained in a mouse with an implanted SERT until death occurs, whereas the same high dose injected into a mouse with no SERT will produce a peak blood alcohol concentration of 425 mg/dl (Fig. 2) plus ataxia or hypnosis lasting for approximately 2 hours (data not shown). This intraperitoneal dose of 3.5 g/kg is thus too high to be used in conjunction with the SERT filled with ethanol. A "low" dose of 1.0 g/kg injected intraperitoneally is adequate for producing tolerance and physical dependence with the SERT, and will initiate a blood alcohol concentration of approximately 200 mg/dl which is maintained for greater than 12 hours with only slight decrement

0036-8075/78/0331-1457$00.50/0

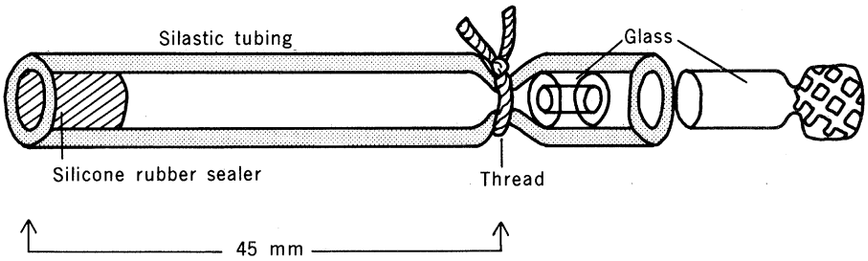

Fig. 1. Diagram of the sustained ethanol release tube (SERT).

(Fig. 2). When SERT's are filled with 95 percent ethanol but no intraperitoneal injections are given, the concentrations of alcohol in the blood remain insignificant, apparently because the ethanol is metabolized as fast as it is released.

In a separate study, we found that if the SERT is refilled every 12 hours and gradually increasing doses of ethanol are given intraperitoneally as tolerance builds up, physical dependence will develop. Mice were maintained on ethanol by refilling the SERT's twice daily with 95 percent ethanol (by volume) and given twice-daily injections (intraperitoneally) of 10 percent ethanol (weight to volume) for 4 to 7 days. The initial injection of 0.5 g/kg (which subjectively produced slightly decreased motor activity in most mice) was increased in subsequent injections to 1.0 g/kg as the mice could tolerate it, such tolerance being indicated by a slight increase in mean body weight and activity of the group on a given day. At midnight on the last day, the SERT's were emptied and a 1.5 g/kg dose of ethanol was injected intraperitoneally into all mice. Average withdrawal seizure scores of 1.5 to 2.0 were obtained after 4 days of exposure to ethanol (*13*), compared to 0.5 for saline-treated mice.

Alcohol has previously been administered to mice on a long-term basis by means of vapor inhalation chambers with (*13*) and without (*14*) metabolic inhibitors such as pyrazole, orally in the diets of alcohol-preferring and nonpreferring strains (*15*), daily by intragastric intubations (*16*), or by multiple around-the-clock injections. Compared with these methods, the SERT has the following advantages: (i) it is inexpensive and simple to make and implant; (ii) it stays in place for at least 14 days because of the pre-tied neck; (iii) it is easy to fill or empty, by means of a 1-ml syringe and flexible PE 50 tubing; (iv) the glass stopper prevents evaporation and is easy to remove in an awake mouse; (v) it is nontoxic, and the 95 percent ethanol is released slowly so that there is no tissue damage, even after 14 days (*17*); and (vi) weight loss after long-term exposure to ethanol with the SERT is minimal and appears to be related to the effects of the mice being isolated (*18*). Caloric control studies with the SERT may thus not be necessary, although studies with higher intraperitoneal booster doses should include isocaloric glucose to match the calories provided by the SERT ethanol plus booster doses of ethanol.

It is possible that other volatile liquids such as acetaldehyde, methanol, tertiary butanol, chloroform, or carbon tetrachloride can be tested by using this implant, since preliminary studies in our laboratories have shown that all of these liquids diffuse through the implant, but at different rates. The tube may also conceivably be made larger for use in rats, rabbits, guinea pigs, or hamsters. Meanwhile, the SERT appears to be a simple and reliable device for the study of ethanol toxicity, tolerance, and physical dependence in mice.

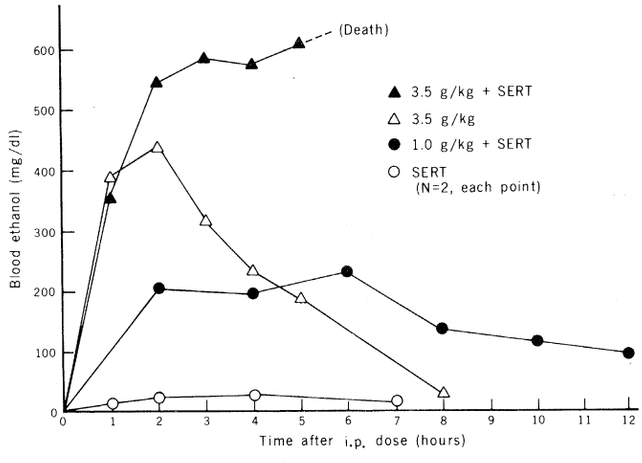

Fig. 2. Maintenance of blood ethanol concentrations in mice with SERT implantations. Ethanol (1.0 and 3.5 g/kg) was injected intraperitoneally (*i.p.*) at time 0; the SERT contained 0.35 ml of 95 percent ethanol (by volume) at time 0. Twelve mice were used for each line, and retroorbital blood samples were collected from two different mice at each point. Blood alcohol was measured by a gas chromatographic micromethod (*19*).

CARLTON K. ERICKSON*
KATHE I. KOCH*
Department of Pharmacology and Toxicology, School of Pharmacy, University of Kansas, Lawrence 66045
CHANDER S. MEHTA
School of Pharmacy, Texas Southern University, Houston 77004
JAMES W. MCGINITY
College of Pharmacy, University of Texas at Austin, Austin 78712

References and Notes

1. D. B. Goldstein, *Life Sci.* **18**, 553 (1976); N. K. Mello, *Psychoneuroendocrinology* **1**, 347 (1976).
2. B. E. Hunter, D. W. Walker, J. N. Riley, *Pharmacol. Biochem. Behav.* **2**, 523 (1974); E. Majchrowicz, *Psychopharmacologia* **43**, 245 (1975); L. A. Pohorecky, *Psychopharmacology* **50**, 125 (1976).
3. D. Lester and E. X. Freed, *Pharmacol. Biochem. Behav.* **1**, 103 (1973).
4. D. B. Goldstein, in *Biological and Behavioural Approaches to Drug Dependence*, H. D. Cappell and A. E. LeBlanc, Ed. (Addiction Research Foundation of Ontario, Toronto, 1975), p. 2.
5. M. A. Jones and G. R. Spratto, *Life Sci.* **20**, 1549 (1977); H. O. J. Collier, D. L. Francis, C. Schneider, *Nature (London)* **237**, 220 (1972); P. G. Goode, *Br. J. Pharmacol.* **41**, 558 (1971).
6. I. Yamamoto, I. K. Ho, H. H. Loh, *Life Sci.* **20**, 1353 (1977).
7. R. J. Hitzemann, H. H. Loh, F. B. Craves, E. F. Domino, *Psychopharmacologia* **30**, 227 (1973); H. S. Huberman, M. S. Eison, K. Smith Bryan, G. Ellison. *Eur. J. Pharmacol.* **45**, 237 (1977).
8. The surgical procedure is as follows: the tube is "sterilized" in 3 percent hydrogen peroxide for 10 minutes and sprinkled with an antibiotic powder (Neosporin). The mouse is anesthetized with ether, and a 0.5-cm cut is made between the ears, and a glass probe inserted along the back under the skin; the SERT is inserted, and the skin is sewn together around the pre-tied neck of the SERT. The mouse is allowed to recover for at least 12 hours before ethanol is administered. Mice implanted with the devices are housed individually to prevent other mice from pulling the stoppers out and chewing the sutures.
9. The SERT's were implanted in female Swiss-Webster mice (ARS/Sprague-Dawley) and filled with 95 percent ethanol, and the amount of ethanol remaining each hour after filling was measured. The SERT is selectively permeable to ethanol; water will not pass in either direction through the wall.
10. H. Wallgren and H. Barry III, *Actions of Alcohol* (Elsevier, Amsterdam, 1970), p. 50.
11. C. K. Erickson, K. I. Koch, C. S. Mehta, J. W. McGinity, *Life Sci.*, in press.
12. A sleep-inducing intraperitoneal dose of ethanol (4.0 g/kg; 20 percent weight to volume) is given to all mice the day before surgery. Those not losing the righting reflex and those sleeping longer than 120 minutes are not used for SERT implantation.
13. Seizures were scored according to the withdrawal rating scale of D. B. Goldstein and N. Pal [*Science* **172**, 288 (1971)].
14. P. J. Griffiths, J. M. Littleton, A. Ortiz, *Br. J. Pharmacol.* **47**, 669P (1973).
15. G. Freund and D. W. Walker, *J. Pharmacol.*

Exp. Ther. **179**, 284 (1971); R. F. Ritzmann and B. Tabakoff, *ibid.* **199**, 158 (1976).
16. M. D. Hammond and C. Schneider, *Br. J. Pharmacol.* **47**, 667P (1973).
17. As judged by a qualified veterinarian using subjective gross observation, slight inflammation was noted 1 day after implantation of either ethanol- or saline-filled SERT's, perhaps because of the destruction of tissue by the probe. The inflammation disappeared by the second day. Fourteen days was the maximum time the SERT's were tested in any one mouse.
18. Weight loss for 12 mice given free access to laboratory chow and water averaged 6.8 percent after 7 days of exposure to ethanol-filled SERT's plus twice-daily intraperitoneal injections. However, a group of control mice (no incisions, no injections) in individual cages showed the same weight loss (mean 6.5 percent) 7 days later.
19. A. E. LeBlanc, *Can. J. Physiol. Pharmacol.* **46**, 665 (1968).
20. We thank P. Lange of Dow Corning for a generous supply of Silastic tubing. The work was supported by research grant AA 01417 (to C.K.E.) from the Department of Health, Education and Welfare.
* Present address: College of Pharmacy, University of Texas at Austin, Austin 78712.

3 October 1977; revised 25 November 1977

Δ^9-Tetrahydrocannabinol: Antiaggressive Effects in Mice, Rats, and Squirrel Monkeys

Abstract. *Δ^9-Tetrahydrocannabinol, the most active constituent of marihuana, decreased species-specific attack behavior in mice, rats, and squirrel monkeys at doses (0.25 to 2.0 milligram per kilogram of body weight) that have no effects on other elements of the behavioral repertoire. Aggressive behavior was engendered in all three species by confronting a resident animal with an intruder conspecific. The present results contrast with the widely held belief that marihuana increases aggressive behavior.*

The link between cannabis and violence goes back at least to the anecdote that fanatic members of a medieval Ismaili sect, called the Assassins (*hashshashin* in Arabic), drank hashish before slaying Crusaders. Contrary to folklore and historical anecdotes, many experimental studies show that acute administration of cannabis extracts of Δ^9-tetrahydrocannabinol (THC), the major psychoactive ingredient in cannabis, reduces aggressive behavior in a variety of situations and animal species (*1*). Yet, the large doses required to decrease such behavior in many of these studies raise the question as to how specific this effect is to aggressive behavior. Moreover, under certain experimental conditions, acute administration of THC or cannabis extract is reported to induce indiscriminate biting and bizarre postures in rats, incorrectly referred to as "aggressiveness" (*2*).

Conclusions from laboratory studies on the effects of THC on aggressive behavior in animals have been limited not only because of high doses, but also because of the large variations in test conditions and in behavior patterns which are considered as "aggression." In the present investigation, aggressive behavior was engendered in mice, rats, and squirrel monkeys by the same experimental manipulations; namely, a stranger animal intrudes into the home cage of a conspecific or a group of conspecifics. Fighting between resident and intruder animals has wide species generality (*3*), and results in intense aggressive behavior including neck bites, attack leaps, threat displays, and defensive, submissive, and flight reactions in the three species studied. Concurrent monitoring of agonistic as well as nonagonistic behavioral elements provides information on which elements of the behavioral repertoire are primarily altered by THC. The present experiments demonstrate that THC, in doses that produce marihuana-like effects in humans, specifically and selectively decreases aggressive behavior in mice, rats, and squirrel monkeys subjected to similar experimental conditions.

In the first series of experiments, male Swiss-Webster albino mice (Zivic-Miller Laboratories, Pittsburgh) were housed individually in Plexiglas cages (28 by 18 by 13 cm) with free access to food and water. After 4 to 6 weeks of individual housing, each resident mouse was confronted in the home cage with an intruder mouse. Intruder mice were housed in groups of five. Resident mice that reliably attacked the intruding mouse during at least three consecutive 5-minute tests without drug were assigned to receive THC (*4*). The THC was injected intraperitoneally once a week in the resident mice ($N = 15$) and, on a second weekly test day, in the intruder mice ($N = 15$). The same resident and intruder mice confronted each other throughout all THC and vehicle tests. Each resident and intruder mouse received the different doses of THC (0.25, 0.5, 1.0, 2.0, and 4.0 mg per kilogram of body weight) in a systematically varied sequence following a Latin-square design. Tests with vehicle only were conducted before and after those with THC. An intruder test lasted 5 minutes after the first attack and was terminated when no attack occurred within 5 minutes. Videotape recordings of all 180 drug and vehicle encounters were analyzed by two experienced observers, each focusing on either resident or intruder mouse. The observers were unfamiliar with the drug treatment of the animals. Latency, frequency, and duration of 11 reliably occurring acts and postures of agonistic and nonagonistic nature were measured (*5*).

In a second series of experiments, male rats of the Sprague-Dawley strain (Zivic-Miller) which attacked an intruding rat in their Plexiglas home cage (28 by 28 by 38 cm) in three consecutive tests were selected for THC administration. Individually housed resident rats ($N = 8$) and group-housed intruder rats ($N = 10$) were maintained at 90 percent of their free-feeding weight and paired for intruder tests once a week, 1 to 2 hours before the daily feeding. As in the tests with mice, the same pair of resident and intruder rats confronted each other during all drug tests. The frequency and duration of the resident and intruder rats' agonistic and nonagonistic behaviors were measured for 5 minutes after the initial attack as described (*6*). Resident rats were injected intraperitoneally with one of four THC doses (0.125, 0.25, 0.5, and 1.0 mg/kg) and with vehicle on alternating tests; in a separate series of experiments, treatments were scheduled in identical sequence for intruder rats. Each rat was subjected to a random sequence of doses.

In a third series of experiments, an intruder monkey was introduced into two separate indoor free-ranging colonies each comprising two or three adult male squirrel monkeys, three adult females, and one infant (*7*). The resident subdominant monkeys of one colony served as intruders into the adjacent colony. The THC, contained in a slice of banana, was administered to either the resident male monkey ($N = 2$) that had attacked the intruder first and most in preceding drug-free tests, or to the intruder monkey ($N = 2$). Drug was administered to the resident and intruder monkeys on alternate test days once a week. An ascending and descending sequence of THC doses (0.25, 0.5, 1.0, and 2.0 mg/kg) and vehicle tests was repeated in each of the two resident and two intruder monkeys. Three observers, two focusing on the two resident males and one on the intruder, recorded the frequency of 15 clearly identifiable agonistic and nonagonistic acts, postures, and displays (*8*).

0036-8075/78/0331-1459$00.50/0