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Subcutaneous Silastic Implants: Maintenance of High Blood Ethanol Levels in Rats Drinking a Liquid Diet[1]

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ERICKSON, C. K., K. I. KOCH AND J. W. McGINITY. *Subcutaneous silastic implants: Maintenance of high blood ethanol levels in rats drinking a liquid diet.* PHARMAC. BIOCHEM. BEHAV. 13(6) 781–786, 1980.—A new subcutaneous form of ethanol exposure in rats is described. The Sustained Ethanol Release Tube (SERT) for rats is similar to an earlier device reported for mice, except that only one refill per day is required. This device, plus an intragastric loading dose for initially raising blood ethanol levels (BEL), is capable of maintaining high BEL for greater than 12 hours. Supplementation of SERT-released ethanol with a Sustacal chocolate-flavored diet with 37% of total energy as ethanol produces high, stable BEL for indefinite periods. Maintenance of such BEL for 9 days is sufficient to cause dramatic withdrawal signs when ethanol exposure is stopped. The method is useful as a model for conveniently and quickly producing physical dependence to ethanol in rats.

Ethanol Sustained release Silastic Liquid diets Sustacal Physical dependence Blood ethanol levels

SINCE heterogenous strains of rats do not routinely drink high quantities of ethanol in drinking water, various methods have been developed for chronically administering ethanol to rats for the purpose of studying alcohol dependence. Certain ethanol preferring and non-preferring rat strains have been developed [7,14], but these animals are expensive to breed and are not yet generally available. Other published methods for producing ethanol dependence in rats include multiple intragastric intubations [12], schedule-induced polydipsia [8], intravenous self-administration [15], intragastric self-administration [16], vapor inhalation [9], liquid diets [1,10] and metabolite-induced ethanol consumption [13]. While all of these methods are useful for special purposes, none of them are capable of producing physical dependence quickly during sustained high blood ethanol levels with only once-a-day intervention in the ethanol administration regimen.

Earlier we reported on a new sustained ethanol release tube (SERT) for producing physical dependence in mice [3,4]. In that model, physical dependence was produced in 4 days, there was no observable tissue damage around the SERT, and there was no significant weight loss related to ethanol exposure. Intraperitoneal doses of ethanol were used to "load" the animal and to produce a desired blood ethanol level which could be sustained with 12-hour SERT refills.

The present study involved the development of a SERT for rats. The device has some characteristics similar to the device for mice. An additional advantage is that the rat SERT requires a refill only once every 24 hours. Dependence can be produced in 9 days with high, sustained blood ethanol levels (BEL).

METHOD

SERT Construction and Implantation

The rat model of the SERT is similar in design to the mouse model reported earlier [3,4]. However, it is larger and has a different stopper (Fig. 1). It consists of a 11.5 cm length of Dow Corning medical grade Silastic® tubing (9.5 mm i.d.×12.5 mm o.d.), sealed at one end with a 1.0 cm Dow Corning silicone rubber plug, and made with a pretied constriction 9.5 cm from the sealed end. (The stopper of a Monoject® blood collection tube, 13 mm × 100 mm, Sherwood Medical Industries, St. Louis, MO, was used. However, any appropriate-sized, lightweight stopper may be used.) The SERT, when full, holds 5.0 ml of 95% v/v ethanol, a volume suitable for the animals used in this study. Although the entire volume of the SERT is not released in 24 hours, the releasing surface is critical for producing optimal blood alcohol levels. Since the diets for the rats are changed daily (see below), it is convenient to refill the SERT at this time.

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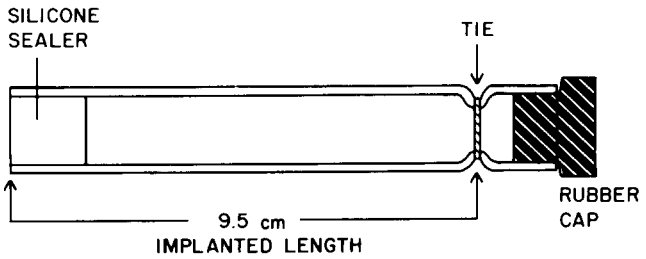

FIG. 1. Diagram of the Sustained Ethanol Release Tube (SERT).

Animals and Implantation

Female SD/ARC rats (derived from Sprague Dawley stock originally obtained from Charles River and bred at The University of Texas) were used for all experiments. They weighed 200–250 g at the time of surgery. These were housed individually after surgery and were maintained on ad lib food (Lab Blox, Wayne) and water during recovery.

The SERTS were "sterilized" in 3% hydrogen peroxide for 10 min. Each rat was lightly anesthetized with ether, and a 1.0 cm transverse cut was made in the neck region. A blunt solid glass probe was inserted through the cut, along the back and under the skin to the tail, to make a straight tunnel. The SERT was then inserted into the tunnel, and the skin was sewn together and tied around the pretied constriction with double ties. The rubber stoppered end thus protruded through the skin at the back of the neck. In this position, the device could be filled using a 5 ml syringe and a 12 cm length of 5 mm o.d. silastic tubing. An antibiotic powder (Neosporin®) was dusted on the SERT and on the cut to reduce bacterial action around the implant, and to prolong implant life. The rat was allowed to recover for 24 hours before filling the SERT with ethanol. Each day during the experiment, the tube was emptied before refilling, so that the concentration of each refill solution was precisely known.

Liquid Diet Formulation

A chocolate-flavored Sustacal® (Mead Johnson, Evansville, IN) liquid diet was used as the sole source of calories and fluid throughout the studies. This diet was used with 37% of total energy as ethanol, and appropriately diluted with water to provide a final concentration of 1 calorie per ml. Control diets contained equicaloric amounts of dextrin in place of ethanol. The liquid diet formula for providing ethanol to rats was as follows:

Sustacal chocolate	315.0 ml
95% v/v ethanol	35.3 ml
Water, enough to make final volume	500.0 ml

Diet was made fresh every 2 days. All glassware to contain the diet was sterilized. Richter tubes were used to reduce spillage. Liquid diet consumption was monitored daily at 0900 hours in the dependence study, or every 2 hours as described in the diurnal consumption study. In the dependence study, control rats (no SERT, no Sustacal) received lab chow and water ad lib.

In Vivo *Studies*

Two methods were used to study the release of ethanol from the SERT *in vivo*. One was a simple measurement of volume of solution remaining in the SERT every 12 hours for 5 days. This was done by carefully withdrawing the solution into a 5 ml syringe, measuring the volume, and then replacing it carefully back into the SERT. Previous studies in our laboratory [4] revealed that this could be done repeatedly with only negligible loss of solution.

The second method involved the measurement of BEL at 4, 7, 12 and 24 hours after filling the SERT, plus intubation of an ethanol loading dose. Tail-tip blood samples of 50 μl into capillary tubes provided blood for analysis. Each sample was placed into a 20 ml vial, heated to 60°C in a rotary turntable waterbath and analyzed by headspace gas chromatography with a Perkin-Elmer Model F40 Multifract instrument. Analytical parameters were: aluminum column, 1.8m ×3 mm with Porapak QS, 80-100 mesh; column temperature, 190°C; carrier (nitrogen) flow, 30 ml/min; injector and detector temperatures, 200°C. External standards were used for calibration.

Diurnal Consumption Study

Because of our interest in using a liquid diet to maintain high BEL as a supplement to the SERT, we examined the rhythmicity in diurnal drinking patterns of rats given chocolate Sustacal plus 37% of total energy as ethanol or dextrin. In these studies, diet consumption and body weights were measured every 2 hours for a total of 48 hours. The light-dark cycle was 0700–1900 hours. Because there was no distinctive pattern of drinking within each period, the 2-hour consumption data were averaged within each period to provide an indication of differences between the drinking patterns of the two groups of rats.

Dependence Study

Various attempts were made to determine the optimal SERT filling times and methods to produce high, stable BEL which would lead to dependence production in most rats. The BEL with the SERT alone were found to be negligible (see Results below), so a "loading" dose was used to set the BEL at a high level which could be maintained over time. Daily IP doses of ethanol were irritating, so oral intubation of 3.5 g/kg (10% w/v ethanol in water) was chosen. In addition, it was found that allowing the SERT-implanted rats to drink a chocolate-flavored Sustacal liquid diet with 37% of total energy as ethanol provided the stable high BEL required for dependence production.

Withdrawal hyperexcitability was scored at the end of the 9-day chronic experiment, using a modification of the method reported by Majchrowicz and Hunt [12]. We have previously described this method in detail [10]:

Signs Measured
- General body spasticity
- Tail tremors
- Caudal tremors
- General body tremors

Scoring for Each Sign
- 0 - absence
- 1 - low intensity
- 2 - moderate intensity
- 3 - severe intensity

Sum of four scores = total score
- Intoxicated rats score 0–4
- Normal rats score 4–6
- Withdrawing rats score 6–12

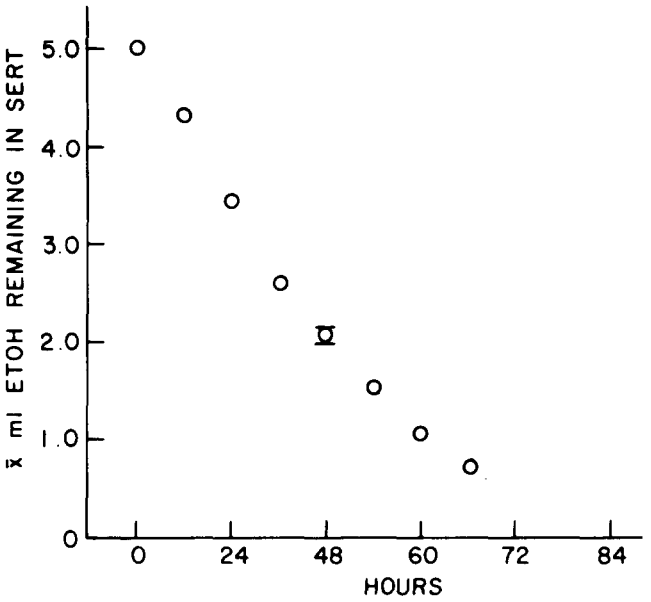

FIG. 2. The *in vivo* release of ethanol from the SERT implanted in rats. The fluid in each SERT was removed, measured and replaced every 12 hours to determine the rate of release. Each point is the mean of 9 measurements. Greatest variation of any point (± S.E.) is depicted at 48 hours.

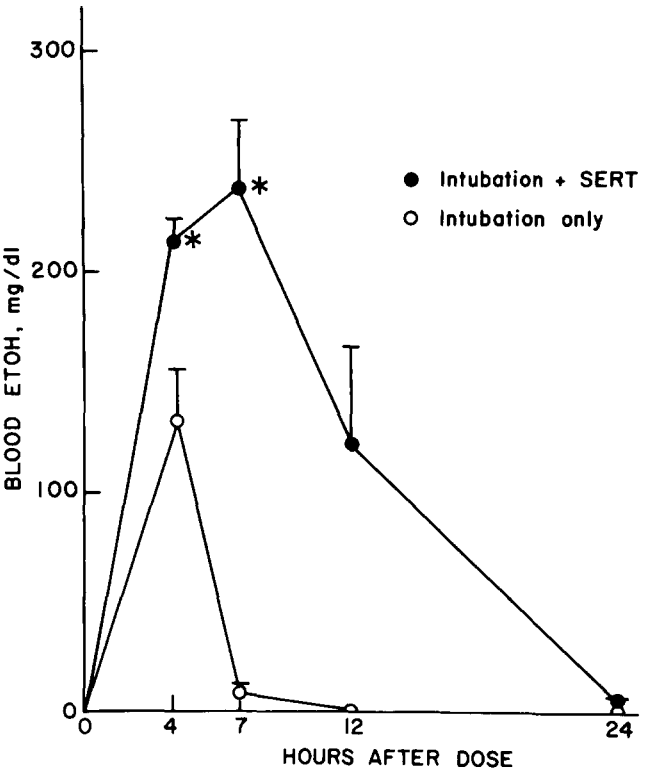

FIG. 3. Enhancement of an orally intubated dose by the SERT. A dose of 3.5 g/kg ethanol in water (10% w/v) was given at time 0. The SERT contained 95% v/v ethanol. Each point is the mean (± S.E.) of blood samples from 6 rats (Intubation + SERT) or 4 rats (Intubation only). Each SERT point is significantly different (*=$p<0.01$) from the corresponding intubation point at 4 and 7 hours.

In order to allow the rats to withdraw from the same BEL, the SERTs of all rats were emptied at 2100 hours on the final day of ethanol exposure. Each rat then received an intragastric intubation of ethanol. The dose of ethanol given was inversely proportional to the degree of intoxication, using the intoxication rating system of Majchrowicz and Hunt [12]:

Intoxication Rating	Oral Dose (g/kg)
Neural	5
Sedated	4
Ataxia 1	3
Ataxia 2	2
Ataxia 3	1
Loss of Righting Reflex	0

Withdrawal signs were scored 13 hours after the 2100 hour oral intubation. Previous studies had shown that signs were maximal at this time [10].

Statistical Methods

In the diurnal consumption and dependence studies, differences among means were analyzed by a one-way analysis of variance. The Student-Newman-Keuls procedure was used to determine significant differences between specific means. In other studies, differences between means were analyzed by the Student *t*-test.

RESULTS

It is known from previous studies such as those with the mouse SERT [4] that ethanol is released through the wall of the Silastic® tube, and that the diffusion of water through the tube is negligible. The loss of fluid volume in the tube over time can therefore be assumed to be due to the release of pure ethanol, and in the case of the rat SERT *in vivo* this release approximates to zero order for the first 48 hours (Fig. 2). In practice, this means that the amount of ethanol released per unit time is constant over this time period regardless of the gradually decreasing volume in the tube. Thus any variations in blood ethanol levels (BEL) over time are probably due to variations in ethanol metabolism in the rat.

From results such as those shown in Fig. 2, we have calculated the mean release rate of ethanol from the rat SERT to be 1.58 ml of 95% ethanol per 24 hours (in 200–250 g female rats), or approximately 250 mg/kg/hour (6 g/kg/24 hours). The metabolic rate of the rat is approximately 260–350 mg/kg/hour [17]; therefore, the SERT releases ethanol at a rate slightly below the metabolic rate. This is the reason that it is necessary to give a "loading" oral dose of ethanol to initially raise the BEL in SERT-implanted rats. The SERT-supplied ethanol then accentuates the effect of the intubated oral dose, as seen in Fig. 3. Although the BEL produced by SERT-released ethanol alone are not depicted in Fig. 3, we have shown in a previous study with the mouse SERT that BEL in such cases are negligible, even when the release of ethanol from the SERT is significantly greater than the metabolic rate of the animal [3,4].

Figure 3 illustrates that the SERT alone is not sufficient for maintaining high BEL for longer than approximately 12 hours. Since we were interested in using a liquid diet regimen to maintain high BEL, we examined the rhythmicity in drink-

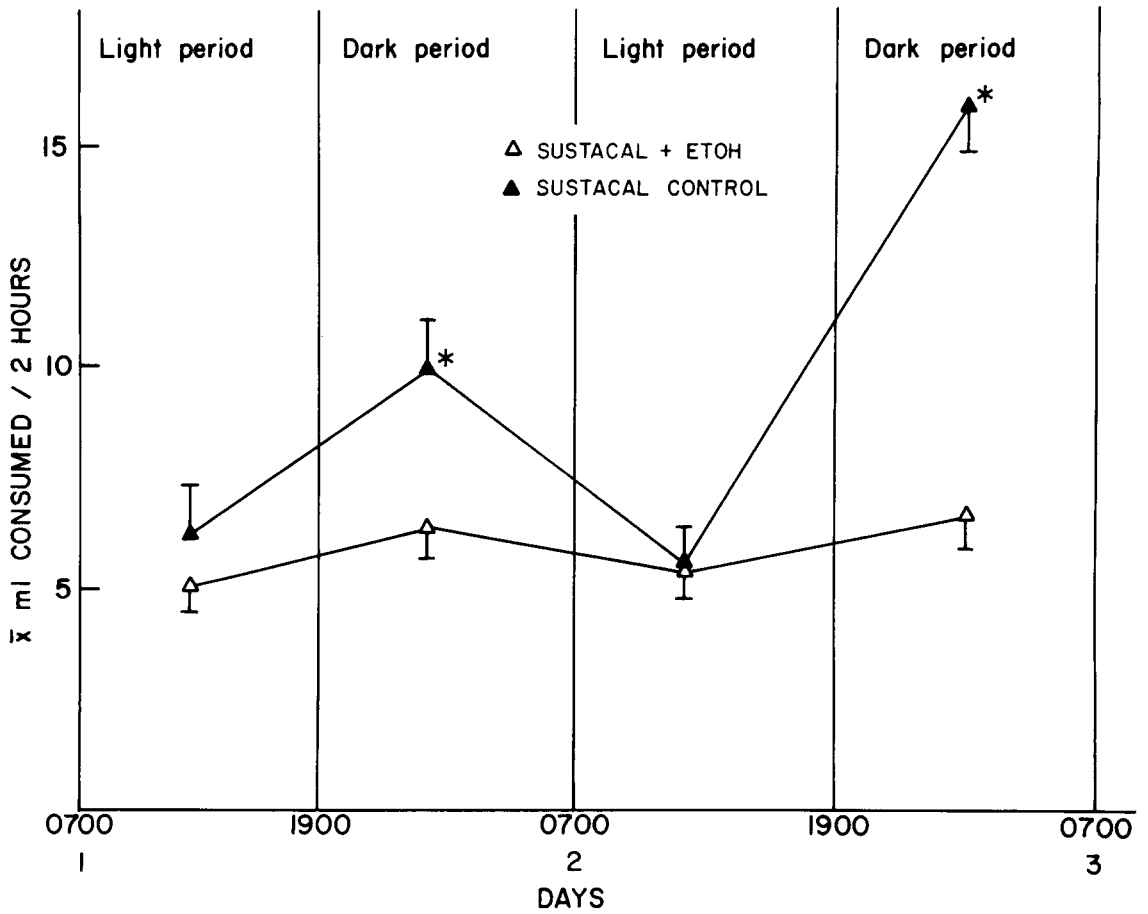

FIG. 4. A diurnal consumption study of Sustacal diets, with and without ethanol. Each point is the mean (± S.E.) of 36 observations, 6 at every 2-hour interval within each period. The two groups are significantly different from each other (*=$p<0.01$) only in the dark periods.

ing patterns of rats given Sustacal plus 37% of total energy as ethanol or dextrin. Figure 4 shows that rats drinking Sustacal alone varied considerably in their consumption of liquid diet, consuming consistently more food during the dark periods than during the light periods. In rats drinking the ethanol-spiked liquid diet, however, there was less variation so that the rats drank almost as much diet during the light periods as during the dark periods. This is probably the reason for the lack of any consistent cycling of BEL in the Sustacal Plus Empty SERT data in Fig. 5.

(In a previous study, we reported that rats drinking Sustacal plus 37% of total energy as ethanol showed "cycling" of BEL which could be stabilized by use of the SERT [2]. Such "cycling" was not confirmed in the larger group of rats used in the present study. However, close examination of individual animal's BEL in the present study revealed some animals in which cycling BEL could be detected for the first 4 days of ethanol exposure.)

In the chronic study (depicted in Fig. 5), the mean daily dietary consumption of ethanol in the SERT-implanted rats was 7.3 g/kg of body weight. The SERT supplied 5.8 g of ethanol/kg in these animals. This exposure resulted in the BEL shown in Fig. 5. The rats with empty SERTs drank more Sustacal each day, but BEL in these rats was lower because of the lack of sustained exposure to ethanol from the SERT.

The results in Fig. 5 and in Table 1 illustrate that the present method is adequate for producing high BEL and a significant degree of physical dependence on ethanol in rats. The dotted line in Fig. 5 represents a hypothetical sustained BEL required to produce physical dependence in animals, as suggested by Goldstein [11], and it is clear that the SERT plus Sustacal with ethanol regimen greatly surpassed this threshold, while the Sustacal with ethanol liquid diet regimen did not always surpass the threshold. The withdrawal scores in Table 1 reflect the difference between these two degrees of ethanol exposure.

As in the intubation model of Majchrowicz and Hunt [12], severe weight loss occurred with high BEL. This appears to be an unavoidable concomitant of high-dose exposure to ethanol, since the rats are so intoxicated most of the time that they do not eat normally. Even the use of a nutritionally adequate diet with the SERT does not significantly reduce body weight loss [5].

DISCUSSION

Goldstein has stated that continuous intoxication is a necessary feature of successful animal models for physical dependence to alcohol [11], and that physical dependence does not persist between binges, when BEL drop to zero or almost to zero. Indeed, the greatest severity of withdrawal

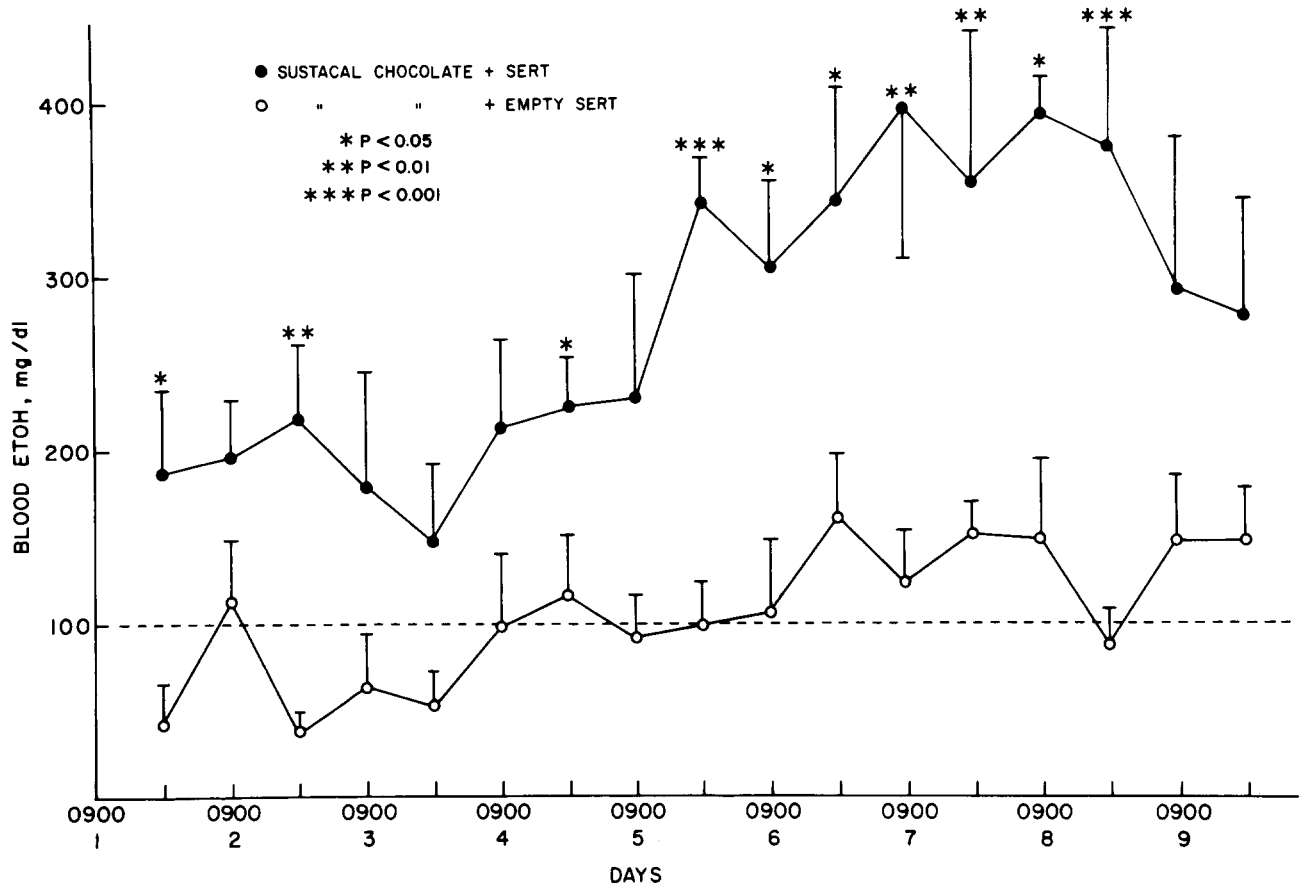

FIG. 5. Blood ethanol levels during the 9-day dependence study. Each point is the mean (± S.E.) of blood samples from 6 rats. The dotted line is a hypothetical sustained threshold for the production of physical dependence.

TABLE 1

BODY WEIGHTS AND WITHDRAWAL SCORES AFTER ETHANOL EXPOSURE FOR 9 DAYS

Group	% Change from starting body weight	Withdrawal score*
Lab Chow control (6)	+ 0.5%	4.2 ± 1.1
Sustacal plus empty SERT (5)	− 4.0%	5.5 ± 0.22
Sustacal plus SERT (5)	−22.0%†	11.0 ± 0.96†

( )=number of subjects.

*Scoring was performed 13 hours after withdrawal. See text for withdrawal scoring system. Numbers are mean raw score ± SE.

†$p<0.001$, Student t-test.

signs is seen when BEL are highest [11], or when doses are "maximum tolerable doses" [12]. Liquid diets, which characteristically produce low BEL, are given to animals over periods of several weeks, after which significant withdrawal signs can be seen [10].

The results in the present study confirm the above general observations and provide another method by which physical dependence can be produced in rats. Unlike the mouse SERT which we described earlier [3,4], the rat SERT leads to significant weight loss during ethanol exposure. This nutritional deficit may accentuate the withdrawal signs ascribed to ethanol, but this should not be construed as a disadvantage of the method if someone wished to study the mechanism of rapid production of physical dependence. We have previously shown that significant withdrawal signs can be induced in the rat without nutritional impairment [10]; therefore, body weight loss, especially in the presence of proper labchow or pairfed controls, is not necessarily a disadvantage.

The advantages of the present method include:

1. convenience of ethanol administration, i.e., the rats must be handled only once a day;

2. sustained high BEL over time, so that maximal physical dependence is produced; and

3. rapidity of physical dependence production, compared to liquid diet-only methods.

The technique of SERT construction and implantation is extremely simple, and one can easily learn to implant a SERT in a matter of minutes. With practice, each daily refill of the SERT will take no more than a minute. Finally, like the mouse SERT, there is absolutely no tissue irritation or destruction around the SERT implant after the slow release of ethanol, because silastic is biologically inert.

While we have also produced physical dependence in rats in 7 days, the withdrawal signs were not as striking as those seen in the 9-day exposure. Variables which appear to be important with regard to the BEL achieved with this method are the size and the metabolic capacity of the rat. For example, some animals died during earlier experiments; BEL were found to be greater than 600 mg/dl. If a rat is too small, or has a low metabolic capacity for ethanol, or is a male [6], BEL will be abnormally high. Conversely, if the rat is too large, or is of another strain that has a higher metabolic capacity than the average Sprague Dawley female, we would expect BEL to be abnormally low and perhaps ineffective in producing physical dependence. It is necessary, then, to determine the size of the SERT required for the male rat, as well as for rats of different ages and strains. Some of these determinations are in progress in our laboratories.

Although we have no data on the reusability of the SERT, there is every reason to believe that the device can be reimplanted in a second rat, in a situation where cost is a factor. However, the device is very inexpensive to construct; therefore, there is probably no need to reuse the devices in most cases.

It has become apparent to us in working with two models of the SERT that the device is probably not adaptable to larger animals, unless custom-designed silastic tubing can be obtained. The wall thickness of the tubing is critical for the release of ethanol, at a rate which approximates the metabolic capacity of the animal. In the mouse, the SERT release rate is greater than the metabolic capacity of the animal, which causes BEL to have a tendency to rise after the initial loading dose. In the rat, the SERT release rate is less than the metabolic capacity of the animal, which causes BEL to fall too rapidly after the initial loading dose, and which leads to the requirement for supplementation with a liquid diet. Theoretically, if a SERT which releases at a rate that matches the metabolic rate of the animal could be made from commercially available silastic tubing, then any loading dose given to the animal would be perfectly maintained by the SERT, and the convenience of the method would remain. Lacking this ideal situation, the SERT is still a valuable method for rapid dependence production in a convenient manner.

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