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# Preference for Alcohol Evoked by Tetrahydropapaveroline (THP) Chronically Infused in the Cerebral Ventricle of the Rat

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C. L. MELCHIOR AND R. D. MYERS. *Preference for alcohol evoked by tetrahydropapaveroline (THP) infused chronically in the cerebral ventricle of the rat.* PHARMAC. BIOCHEM. BEHAV. 7(1) 19–35, 1977. The voluntary preference for ethyl alcohol in Sprague-Dawley rats was determined over 12 days with water as the alternative fluid. The alcohol solutions offered to the animals were increased systematically in concentrations from 3 to 30%, according to a three-bottle, two-choice technique. Tetrahydropapaveroline (THP), a tetrahydroisoquinoline derivative, was infused repeatedly into the lateral cerebral ventricle of each rat through a guide tube implanted chronically. The metabolite was dissolved in a CSF vehicle, and infused in a volume of 1.0 μl every 15 min or 4.0 μl every 30 min around the clock, for the entire 12-day period of alcohol-water self-selection. Within 3 to 6 days of the start of infusion, extraordinary amounts of alcohol were consumed which ranged as high as 8 to 17 g per kg per day. Both the racemic mixture of THP and the S-(-)-THP isomer exerted this alcohol-inducing effect, when they were infused chronically in a range of doses from 100 picograms/μl to 1.0 μg/μl. Control intraventricular infusions of CSF according to the same regimen had no effect on alcohol preference. The excessive intake of alcohol during the intraventricular infusions of THP persisted long after the cessation of the infusion regimen, i.e., during retests carried out at one, six and nine months' intervals. Further, when THP-treated rats were offered a simultaneous choice of a palatable solution of saccharin together with alcohol, they continued to drink large volumes of alcohol. The 24 hr patterns of fluid intake, as registered continuously by a drinkometer, revealed that alcohol drinking was typically massed within two to four bouts during the night-time interval. During this period, the blood alcohol level reached concentrations as high as 0.2%. Withdrawal-like symptoms including wet-dog shakes, elevated tail, whisker twitching and occasional convulsive episodes, were also observed in the THP-infused rats. These findings provide support for the hypothesis that an alkaloid metabolite, which may be formed in both the brain and periphery, is involved in the mechanism underlying the pathological and sustained drinking which is characteristic of the disease state of alcoholism.

Alcohol preference pattern  Tetrahydroisoquinoline (TIQ)  Chronic cerebroventricular infusion  Tetrahydropapaveroline (THP)  Limbic system and alcoholism  Ethanol self-selection  Withdrawal symptoms  Blood alcohol levels  Drinking of alcohol

IN 1970, two independent groups of investigators hypothesized that certain condensation products of biogenic amines and aldehydes may be formed following the ingestion of ethyl alcohol [3,8]. Special attention was focused upon one of these substances, tetrahydropapaveroline (THP) which condenses from dopamine and dopaldehyde. Because this alkaloid is a precursor of morphine in the opium poppy, *Papaver somniferum* [20], THP could constitute the vital biochemical link between the addiction to morphine and to alcohol [8]. Moreover its formation could underlie the neurohumoral mechanism of addiction to alcohol.

The initial *in vitro* studies of Holtz *et al.* [17] revealed that THP is formed peripherally, when liver mitochondria are incubated with dopamine. Similarly, THP is detectable in the homogenate of the rat's brain stem if incubated with $^{14}$C-dopamine and either ethanol or acetaldehyde [8, 9, 46]. *In vivo* not only is THP synthesized in the liver [14], but an appreciable amount of the metabolite is excreted in the urine of the Parkinsonian patient who is undergoing L-DOPA therapy and simultaneously ingests alcohol [44]. Although these studies indicate that THP can be produced peripherally, the experiments of Turner *et al.* [46] demonstrate the *in vivo* synthesis of THP in the brain of the rat. Using the technique of mass fragmentography, they identified THP in quantities ranging from 2.0 to 15.0 ng per g of brain tissue following the chronic administration of L-DOPA orally and in concentrations of 10.0 to 25.0 ng per g of brain tissue when alcohol was also given.

With respect to its functional role, THP possesses β-sympathomimetic activity, as first documented by Laidlaw in 1910 [21] and confirmed by other workers more recently [39]. THP may also inhibit metabolic processes in neuronal tissue which include the uptake of catecholamines [1], dopamine-o-methylation [4,13], serotonin degradation by monoamine oxidase [4,13] as well as the dopamine-stimulated production of cyclic AMP by the dopamine-type adenylate cyclase [45]. Nevertheless, a major question today revolves about the actual relationship of THP to alcohol drinking, withdrawal, and the addictive

mechanism in general. Recently, we reported that the chronic infusion of THP into the cerebral ventricle of the rat causes the animal to select and drink solutions of alcohol even at concentrations which are gustatorily noxious [35]. This finding has provided the first evidence of a functional link between an amine condensation product and the pathological drinking of alcohol.

The purpose of this investigation was to characterize further several notable facets of THP's central action on alcohol drinking of the rat. The present experiments were undertaken, therefore, as follows. First, THP was infused in a wide range of doses according to a chronic regimen in an attempt to establish a dose-response relationship with respect to alcohol intake. Second, the S-(-)-isomer of THP was infused in the same way in order to examine the question of stereospecificity of THP. Third, the drinking of alcohol was tested when other, more palatable fluids were made available to the rat in which THP was infused intraventricularly. Fourth, symptoms of withdrawal-like behavior and the induction of seizures in the THP infused rat were studied. Fifth, the duration of action on alcohol drinking of THP given by the ventricular route was ascertained. Finally, alcohol preference following daily intraperitoneal injections of an efficacious central dose of THP was determined.

## METHOD

### *Animals*

Male rats of the Sprague-Dawley strain weighing 350 to 500 g were housed individually in an open-topped cage which was 60 cm high and maintained under a 12-hr light-dark cycle at a temperature of 21 to 23°C. Powdered Wayne Lab Blox was provided ad lib. to each rat from a metal cup dispenser affixed to the floor of the cage.

### *Alcohol Preference Determination*

Three calibrated 100 ml Kimax drinking tubes were positioned equidistantly on the front of each animal's cage. One tube contained a solution of ethyl alcohol which was increased in concentration on each day of the 12-day test sequence as follows: 3, 4, 5, 6, 7, 9, 11, 13, 15, 20, 25 and 30 percent. Each volume/volume solution was prepared with 95 percent ethanol and deionized water. A second tube contained only water whereas the third tube was empty. These tubes were rotated each day according to a predetermined random sequence to prevent the development of a position habit [33]. By systematically raising the concentration of alcohol every day, a curve of preference-aversion could thereby be obtained. Body weight and food intake of each rat were recorded every other day.

### *Surgery*

Each rat was anesthetized with 30 mg per kg of sodium pentobarbital and then placed in a stereotaxic instrument in the DeGroot orientation. After a bur hole was drilled in the skull at AP 5.8, a guide cannula was positioned 1.5 mm off mid-line and lowered to a depth of 3.0 mm below the dura. The guide cannula consisted of an 8 mm length of 20 ga thin-wall stainless steel tubing inserted into a threaded teflon pedestal, as modified after Khavari [19]. A 27 ga stainless steel injector needle was inserted through the pedestal and guide tube, and patency with the lateral ventricle was ascertained with an infusion by gravity flow of an artificial cerebrospinal fluid (CSF). Then, cranioplast cement was packed around the guide tube pedestal and bone screws so as to hold the cannula assembly firmly in place. A 27 ga stainless steel stylet of the same length was inserted in the guide tube to prevent the guide from becoming obstructed.

### *Chronic Infusion Procedure*

A 27 ga injector needle was friction-fitted within a teflon hub, so as to extend 1.0 mm beyond the guide tube. The hub-needle assembly was held in place by a cap which was screwed onto the guide tube pedestal [19]. Since the needle hub could turn freely inside the cap, the animal had complete freedom of rotational movement. A length of 20 ga polyethylene (PE) tubing (PE 20) was forced onto the injector needle and run from this Khavari swivel [19] to a swivel-bolt pulley suspended above the animal's open-topped cage. A metal weight of approximately 30 g was positioned on the tubing between the pulley and the side of the cage. To prevent the rat from reaching the tubing and pulling it into its mouth, a 6 cm length of 4.5 mm O. D. Tygon tubing was placed over the hub and tubing. The other end of the 20 ga PE tubing was connected to a 1.0 ml glass tuberculin syringe. A custom-built pump, designed to hold 12 infusion syringes simultaneously, was programmed to deliver an injection of a volume of either 1.0 $\mu$l over 13 sec every 15 min or 4.0 $\mu$l over 60 sec every 30 min.

Either tetrahydropapaveroline hydrobromide (THP) or S-(-)-tetrahydropapaveroline hydrochloride (S-(-)-THP) was dissolved in an artificial CSF [31], which consisted of 7.46 g NaCl, 0.19 g KCl, 0.14 g anhydrous $CaCl_2$ and 0.19 g $MgCl_2 \cdot 6H_2$ per liter of glass-distilled water. To retard the degradation of the condensation product, the pH of the solution was lowered to 3.8 by the addition of 0.1 mg/ml of ascorbic acid. Each solution was prepared in pyrogen free glassware and passed through a 0.22 $\mu$ Swinnex millipore filter. So that the PE tubing could be flushed with CSF and reloaded with a freshly prepared solution of THP, the injector needle was removed from the pedestal base for a period of approximately one hr or less on each day. During this interval, all needles were resterilized in 70% alcohol.

Infusions of THP were made into the ventricle of each animal, according to the individual round-the-clock schedule, for two days prior to the beginning of the 12-day alcohol preference test. This regimen of THP administration was then maintained during the entire course of the sequence. Again, intakes of food and fluid were recorded every day, and the animals were weighed every other day.

To ascertain the long-term effect of THP, preference for alcohol of four animals was retested one month after the start of an alcohol test sequence. During this time, only the CSF vehicle was infused according to the previous injection procedures. Three rats were retested again for their preference for alcohol at intervals of three, six and nine months, with no further infusions given.

To test the effect of THP in the absence of exposure to alcohol, 4.0 $\mu$g per 4.0 $\mu$l of THP were infused intraventricularly every 30 min for two weeks. At the conclusion of this interval, preference for alcohol was determined according to the same 12-day test sequence. During this period, CSF constituted the infusate, after which all intraventricular infusions were terminated. Then, during four successive periods, constant concentrations of alcohol

were offered as a choice against water in the following order: 15% for 10 days, 7.5% for the next 10 days, 15% for the next 14 days and 30% for the last 12 days.

In order to specify the time during each 24 hr day-night cycle when a given rat drank an alcohol solution, drinkometer records for individual animals were collected. In this case, a given drinking tube and the grid floor of the cage were wired to an automatic BRS Foringer sensor circuit. Each time the rat licked the alcohol solution, a pulse was relayed to a Sodeco Print-out counter with total licks registered once per hour. Drinkometer records reflecting alcohol intake were collected for rats in which THP was infused during the presentation of: (1) 15–30% alcohol concentrations, offered at the end of a 12-day alcohol test sequence, or (2) 20% alcohol versus water for three days. Records were also taken during a post THP alcohol test sequence in which the control CSF was infused.

Two methods of measuring alcohol consumption were employed. By calculating the ratio of the volume of alcohol solution consumed to the daily fluid intake, a measure of alcohol preference was obtained. The second measure provided an indication of the total amount of alcohol ingested. The weight of alcohol consumed in grams was divided by the weight of the animal in kilograms.

*Taste Tests*

In five rats in which 0.2 or 1.0 μg per μl of THP were infused intraventricularly, a second 12-day alcohol preference sequence was initiated. In this case, one drinking tube contained the same range of concentrations of alcohol as that used previously; however, each of the alcohol solutions was mixed with $2 \times 10^{-5}$ M sodium saccharin. The second drinking tube was filled with water. The third tube, which is normally empty, contained only the solution of saccharin alone. Throughout this period, the chronic intraventricular infusions of THP were continued.

In a different experiment, rats were maintained throughout on a similar infusion schedule of 4.0 μg per 4.0 μl infusion of THP. But in this case the concentration of alcohol was held constant at 20% for three days. Then for the next four days, the rats were offered a choice between 20% alcohol in one drinking tube, an isocaloric solution (0.3 g per 1.0 ml) of Sustagen (Mead Johnson) in the second tube, and water in the third tube. The alcohol solution was then reduced to 10% and the Sustagen solution lowered to a corresponding isocaloric value (0.15 g per 1.0 ml) over the next three days.

*Blood Alcohol Determinations*

After a 25 μl aliquot of blood was obtained from the tip of the rat's tail, the level of alcohol in the sample was determined by means of the modified [26] enzymatic assay of Roos [41]. Determinations were made in samples taken during selected times throughout the alcohol test sequence in five animals in which 0.02 μg per μl of THP was infused intraventricularly. For example, blood was obtained at 8 p.m., 8 a.m., or during peak drinking periods, as reflected by the drinkometer records, in the early morning hours between midnight and 3 a.m.

*Behavioral Observations*

The animals were observed periodically throughout the day and night for signs of behavioral or motor alterations. The presence or absence of signs of intoxication as denoted by Majchrowicz [25] or withdrawal-like behavior [18,25] was recorded daily. Two tests for proneness to audiogenic seizures were conducted: (1) jingling keys above the head of the rat [11], or (2) placing the rat in an enclosed chamber and sounding a 122–126 dB alarm bell for 90 sec [12].

*Verification of Ventricular Infusion*

To determine whether the infusate actually entered the ventricular system of each rat, a dye substitution method was employed to validate the chronic procedure [32]. Upon completion of the experiment, the animal was given an overdose of sodium pentobarbital administered intraperitoneally. A volume of 2.0 to 10.0 μl of India ink was injected through the same needle as that used for the chronic infusion. An injection volume somewhat larger than that used during actual THP or CSF infusions was given, in some cases, for greater ease of visualization of the ink. The brain was removed from the skull with small spatulas and carefully dissected.

The extent of the diffusion of the ink was determined by exposure of the ventricular cavities by parasagittal incisions through the superficial cortex. Cuts were made in the rostro-caudal orientation approximately 3.0 mm deep and 1.5 mm off midline [32]; the third and fourth ventricles were visualized similarly following their exposure with a mid-sagittal incision from the corpus callosum to the base of the lower brain-stem. If evidence of dispersion of the ink was not clear, the animal's record was considered separately from those in which the ink was found in the ventricles.

*Acute Injection of THP*

In five animals prescreened for their alcohol preference on the 3 to 30% sequence, a single acute intraventricular injection of 40.0 μg of the free base of THP was given in a volume of 20 μl. By performing a surgical procedure similar to that described previously [27,34] the injection was made by gravity flow after an injector needle was lowered into the ventricle. Following the injection, the incision was closed with a wound clip. A four-day period of recovery elapsed prior to the start of an alcohol test sequence.

*Peripheral THP Treatment*

Twelve rats were divided into two groups of six rats each. An intraperitoneal injection of 64 μg of THP in a volume of 32 μl of sterile saline was given to each animal of one group three times a day, at 8 a.m., 4 p.m. and 12 p.m. In the remaining six animals, the same volume of saline was injected at the same time. This schedule of peripheral injections was started 2 days before a 12-day, 3 to 30% alcohol preference sequence and maintained throughout.

Two weeks after the completion of the test sequence, the same procedure was repeated but the injection regimen of the two groups was reversed. That is, the animals that received THP during the first alcohol sequence were given 32.0 μl of saline three times a day and, conversely, the rats treated with saline during the first sequence were given 64.0 μg of THP in 32.0 μl of saline three times a day during the second.

## RESULTS

In confirmation of previous findings [35], the chronic

TABLE 1

PROPORTION OF ETHANOL TO TOTAL FLUID INTAKE AND Gm OF ETHANOL PER Kg OF BODY WEIGHT CONSUMED BY EACH GROUP OF ANIMALS IN WHICH THP WAS INFUSED CHRONICALLY IN THE CEREBRAL VENTRICLE. THE RESULTS ARE EXPRESSED IN TERMS OF FIRST HALF (3–9%) AND SECOND HALF (11–30%), AS WELL AS THE ENTIRE (3—30%) ETHANOL TEST SEQUENCE

| THP Dose ($\mu$g/$\mu$l) | N | Proportion 3–9% (1st 6 days) | Proportion 11–30% (2nd 6 days) | Proportion 3–30% (Overall) | g/kg 3–9% (1st 6 days) | g/kg 11–30% (2nd 6 days) | g/kg 3–30% (Overall) |
|---|---|---|---|---|---|---|---|
| 2.0 | 5 | 0.85 | 0.55 | 0.70 | 3.5 | 4.9 | 4.2 |
| 1.0 | 3 | 0.72 | 0.71 | 0.72 | 5.1 | 8.4 | 6.8 |
| 0.2 | 3 | 0.68 | 0.39 | 0.53 | 3.0 | 4.4 | 3.7 |
| 0.02 | 4 | 0.94 | 0.76 | 0.85 | 4.9 | 8.4 | 6.6 |
| CSF Control | 4 | 0.15 | 0.06 | 0.10 | 0.3 | 1.0 | 0.6 |
| Nonventricular Control | 7 | 0.27 | 0.03 | 0.15 | 0.7 | 0.4 | 0.6 |

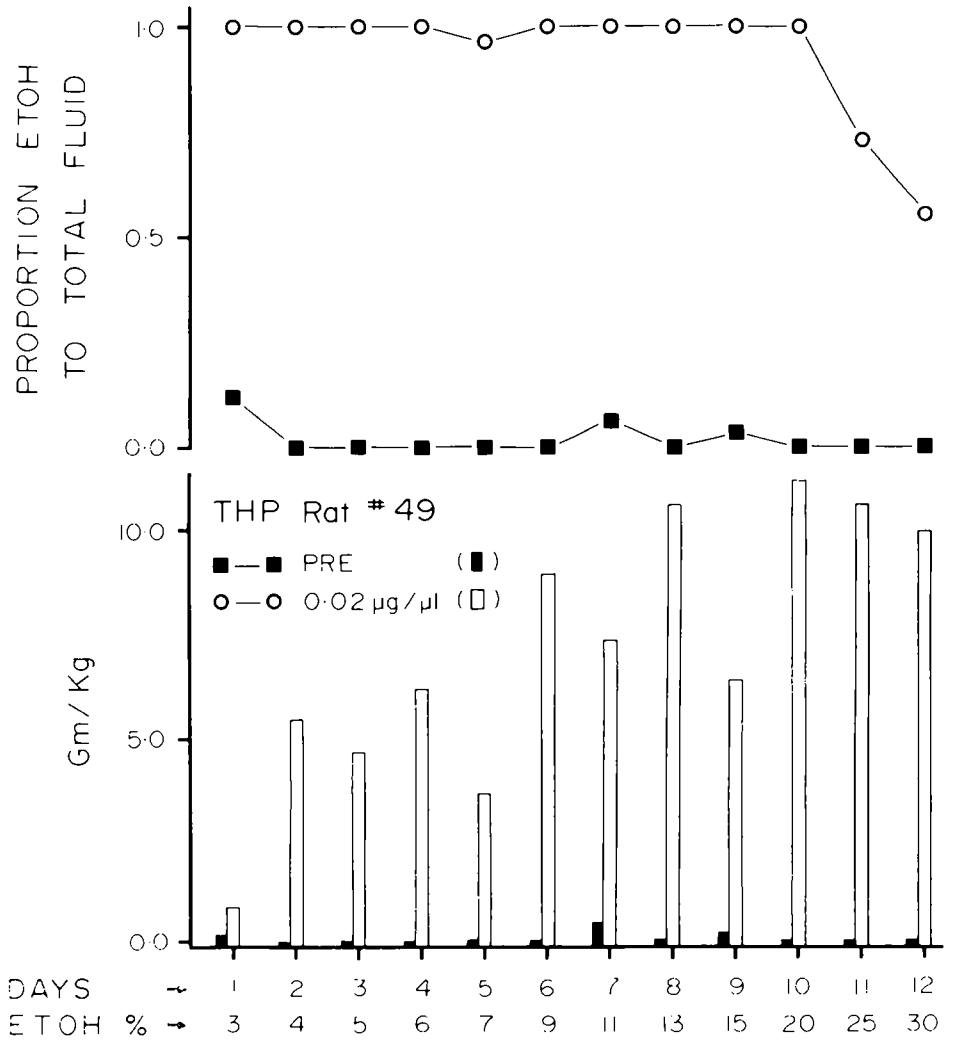


FIG. 1. The proportion of alcohol to total fluid intake (TOP) and g alcohol per kg body weight (BOTTOM) consumed for each concentration of alcohol offered before (PRE) and during the intraventricular infusion of 1.0 µl every 15 min of 0.02 µg of THP in rat No. 49.

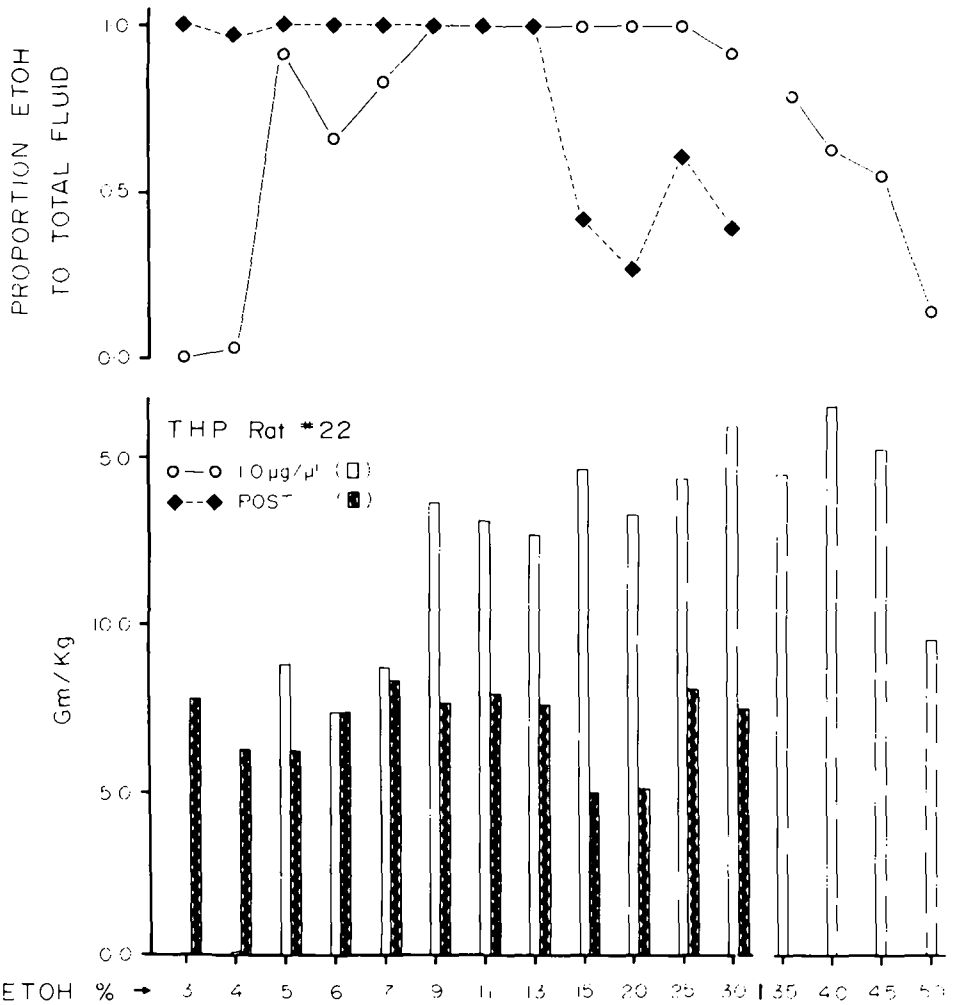


FIG. 2. The proportion of alcohol to total fluid intake (TOP) and g alcohol per kg body weight (BOTTOM) consumed for each concentration of alcohol offered to rat No. 22 during the intraventricular infusion of 1.0 μl every 15 min of 1.0 μg of THP and during the succeeding infusion of CSF (POST).

infusion of THP into the cerebral ventricle induces the rat to drink abnormally large amounts of alcohol, both in terms of solution preference and overall intake. As indicated in Table 1, when THP is infused into the ventricle, in all of the doses ranging from 0.2 to 2.0 μg per μl, the animals select more than half of their mean daily fluid intake in the form of the alcohol solution offered. This preference and intake of alcohol were significantly greater than that observed for those animals in which the CSF control vehicle was infused ($p<0.01$, Newman-Keuls test) or in which the THP infusate did not enter the ventricle but rather diffused over the cerebral cortex ($p<0.01$). Even during the second half of the sequence when the gustatorially aversive concentrations of alcohol from 11 to 30% were offered, these animals continued to prefer alcohol over water. For example, THP infused in both 1.0 and 0.02 μg doses caused an intake of 8.4 g per kg per day during this latter half of the test sequence. This is in sharp contrast to the 1.0 and 0.4 g amounts of the two control groups (Table 1).

Figure 1 (TOP) illustrates the remarkable shift in alcohol preference in a rat which, somewhat unusually, had rejected alcohol even in the lowest of the concentrations offered; yet during the chronic infusion of 0.02 μg per μl, this animal consumed its entire fluid intake as alcohol until the 11th and 12th days of the sequence. A corresponding, ever-increasing rise also occurred in the g of alcohol ingested by the rat. This is illustrated in Fig. 1, (BOTTOM) which shows that the THP-infused animal's intake of 13%, 20%, 25% and 30% alcohol exceeded 10 g per kg per day. The high intakes of some rats surpassed the 12 g per kg level of alcohol that may be metabolized over the course of a day [22]. As shown in Fig. 2, one representative rat consumed more than 12.6 g per kg of alcohol on each of the last seven days of the 12-day alcohol sequence. Although the average g per kg intake for the entire test period was 10.2, the mean for the last six days, when the highest concentrations were offered, was 14.0. Because of this, an additional four days was added to the normal sequence in which 35%, 40%, 45% and 50% concentrations of alcohol were presented according to the same free-choice paradigm (see Fig. 2). At the first three of these concentrations, the rat drank 14.5, 16.6 and 15.3 g per kg, respectively, on each of the three days. Only at the 50% concentration did the rat's total fluid intake decline; even then 9.6 g per kg were ingested.

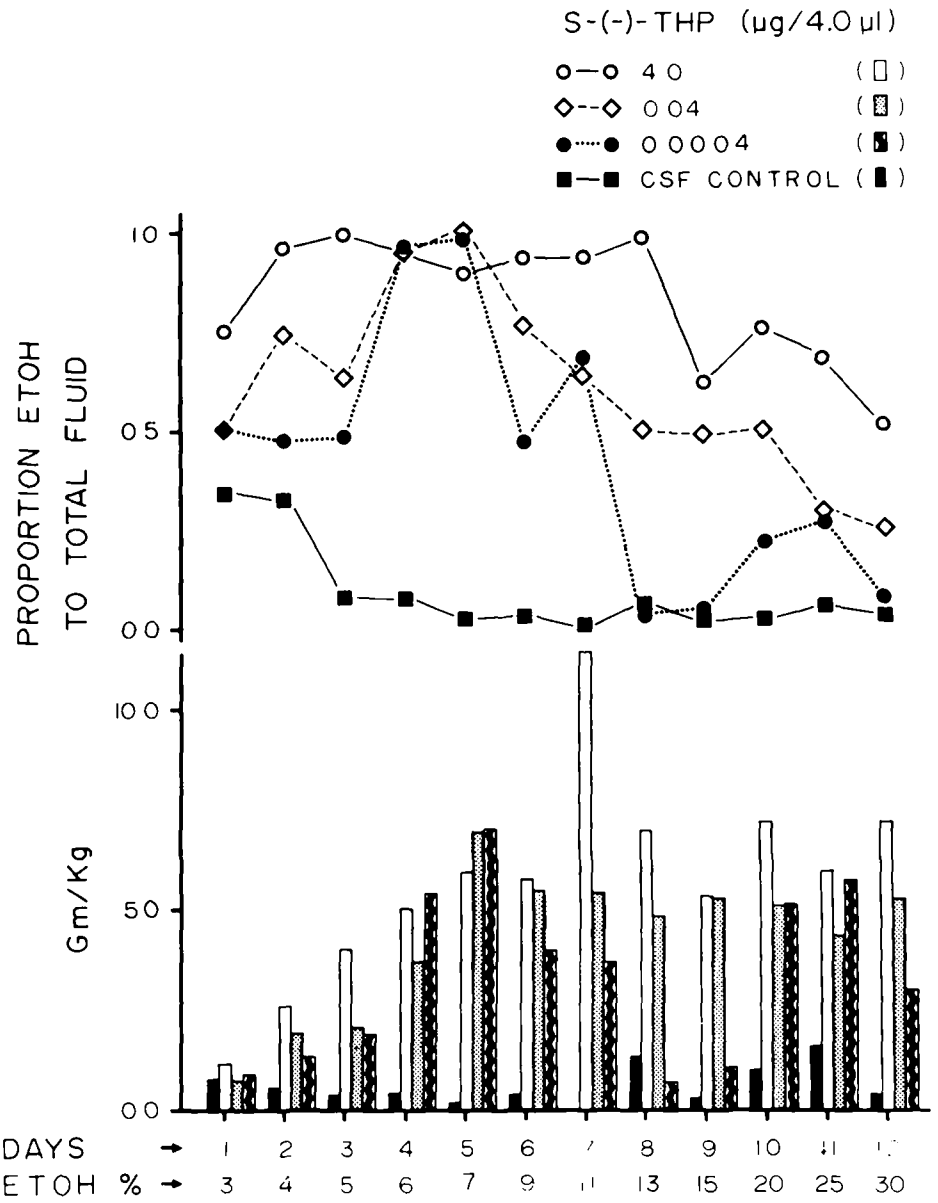


FIG. 3. The proportion of alcohol to total fluid intake (TOP) and g alcohol per kg body weight (BOTTOM) consumed for each concentration of alcohol offered during the intraventricular infusion of 4.0 µl every 30 min of CSF (N = 3) or 4.0 µg (N = 2), 0.04 µg (N = 2) or 0.0004 µg (N = 2) of S-(-)-THP.

Overall, these intake levels are the highest ever reported for the rat. The proportion of alcohol to total fluid intake (Fig. 2 TOP) was concordant with the large volume of alcohol ingested, namely 0.79, 0.63, 0.55 and 0.15, respectively, for the four additional test days. Following a three day interval in which the rat was offered only water, THP was replaced by the control CSF vehicle as the infusate. The rat was then retested on the 12-day alcohol preference sequence. As presented in Fig. 2, the rat continued to drink excessive amounts of alcohol averaging 7.0 g per kg during this second alcohol test sequence. It is important to note that the g per kg values were almost constant throughout the sequence (Fig. 2 BOTTOM) in contrast to the rising intake which occurred during the first test.

*Infusion of the S-(-)-THP Isomer*

A similar increase in both preference for and consumption of alcohol occurred in rats in which 4.0 µg per 4.0 µl of the THP isomer, S-(-)-THP, were infused. Whereas the mean proportion rose from 0.20 before infusion to 0.83 during infusion, the intake of alcohol increased above the average control level of 1.0 g per kg to 5.7 g per kg per day.

Figure 3 illustrates the proportional shift (TOP) and the g intake change (BOTTOM) observed in these animals in which either CSF or one of three doses of the isomer was infused. Even with much lower doses of S-(-)-THP, a somewhat similar increase in alcohol consumption was nevertheless noted. A dose which was one-hundred fold less (0.04 µg) increased the mean proportion of alcohol to total

TABLE 2

THE PROPORTION OF ETHANOL TO TOTAL FLUID INTAKE AND Gm ETHANOL PER Kg BODY WEIGHT CONSUMED BEFORE CHRONIC INFUSION (PRESCREEN CONTROL), DURING INFUSION OF THP AND AT INTERVALS AFTER THE INFUSION OF 4.0 μg/4.0 μl OF THP (N = 3)

| Test | Proportion | Gm/kg |
|---|---|---|
| Prescreen Control | 0.34 | 1.4 |
| During infusion | 0.79 | 5.5 |
| 1 month post | 0.60 | 4.7 |
| 3 months post | 0.66 | 3.4 |
| 6 months post | 0.82 | 3.9 |
| 9 months post* | 0.77 | 4.0 |

*N = 2 since one animal died.

fluid intake from a control value of 0.05 to 0.60 and the g intake from a control level of 0.3 g per kg to 4.3 g per kg per day. A dose of only 0.4 nanograms of the isomer, infused intraventricularly, likewise caused an elevation in alcohol preference, from a proportion of 0.13 to 0.43 and from 0.5 g per kg to 3.3 g per kg per day in terms of actual intake. This dose is one ten-thousandth of the highest dose, and may begin to approach the lower limit of THP's efficacy, since the proportion and g per kg intake measures were significantly different from those of the rats given the 4.0 μg dose ($t$ = 4.99 and 3.28, respectively, $p < 0.01$). Of the animals given the lowest dose of S-(-)-THP, one decreased its food and fluid intake following a nasal discharge, whereas the other was polydipsic (mean intake of 72 ml per day).

*Duration of THP's Effect*

In rats in which 4.0 μg per 4.0 μl of THP were infused chronically into the ventricle, the mean proportion of alcohol to total fluid intake increased from a control level of 0.34 prior to surgery, to 0.79 during the infusion of the alkaloid. As shown in Table 2, the preference for alcohol remained much higher than that of the control test at one-month, three-month, six-month and nine-month post-infusion intervals. Likewise, the actual intake of alcohol increased on the average from three to four-fold with an average of 4.0 g per kg consumed per day. The actual preference pattern for a group of animals given a dose of 4.0 μg of THP per infusion is presented in Fig. 4. One month after the discontinuation of the THP infusions, the preference pattern in terms of proportion of alcohol to water was virtually identical. Even six months later the preference persisted until the 15% concentration, recovered at the 20% level, and then diminished at the 25% and 30% concentrations of alcohol offered. The intake of alcohol by this same group is correspondingly illustrated in Fig. 5. Of considerable interest is the fact that the amount of alcohol consumed virtually stabilized at the 4.0 to 6.0 g per kg level once the 7% concentration had been reached during this preference sequence, six months after the last infusion of THP.

In one rat, an exceptional pattern of preference was observed in a pre-infusion control sequence. As shown in Fig. 6, this rat consumed as much as 5.0 g per kg at the 6 and 11% concentrations. During the infusion with THP at the 4.0 μg dose, the gm intake ranged between 8.0 and 10.0 g per kg per day. However, on the postalcohol preference sequence given one month following the last intraventricu-

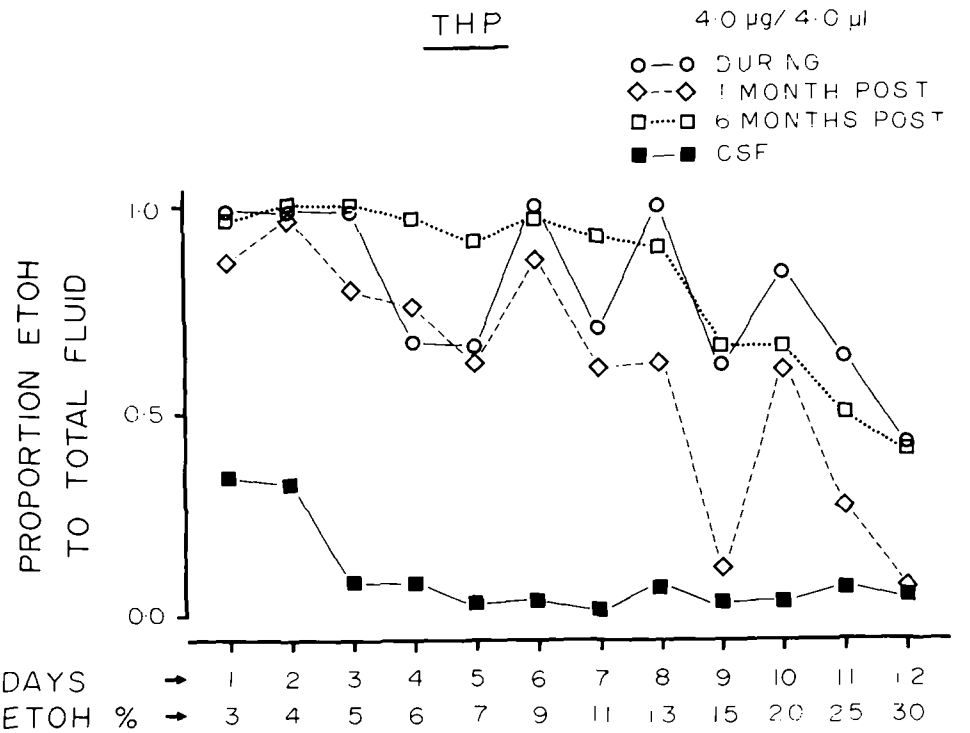


FIG. 4. The proportion of alcohol to total fluid intake consumed for each concentration of alcohol offered during the intraventricular infusion of 4.0 μl every 30 min of CSF (N = 3) or 4.0 μg of THP, and one month and six months after the infusion of THP (N = 3).

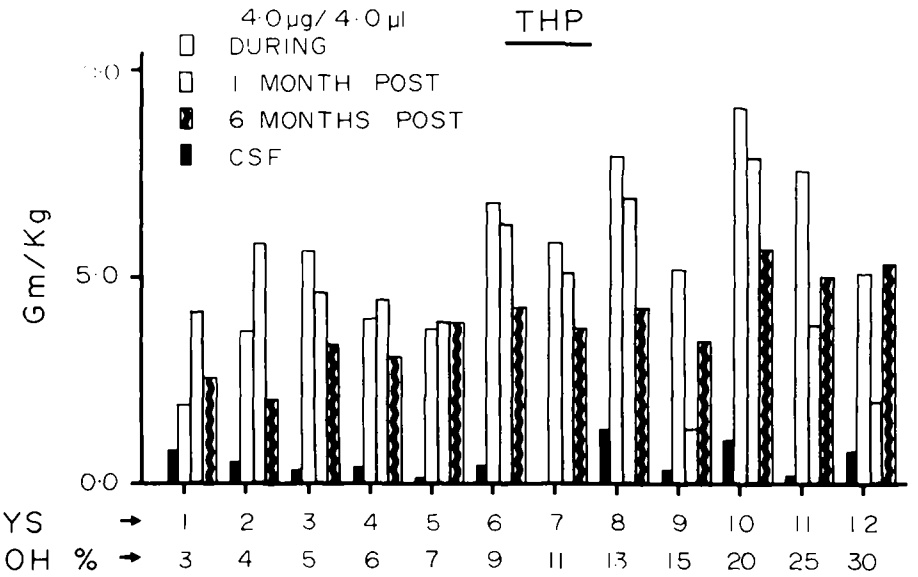


FIG. 5. Gram alcohol per kg body weight consumed for each concentration of alcohol offered during the intraventricular infusion of 4.0 µl every 30 min of CSF (N = 3) or 4.0 µg of THP, and one month and six months after the infusion of THP (N = 3).

lar infusion, again a steadily-rising intake of alcohol occurred in terms of the gm per kg measure even though the proportional values declined at the 15% concentration and solutions thereafter.

That alcohol drinking must be paired with the exposure of the rat's brain to alcohol is not the case. In two animals, a dose of 4.0 µg per 4.0 µl of THP was infused every 30 min for 14 days before the first presentation of an alcohol preference test sequence. As shown in Table 3, the proportion as well as g per kg intakes were very high during the 3 to 30% preference test over the 12-day period. Following this, both rats were offered water and a constant concentration of 15% alcohol. In both cases, the intakes exceeded 6.0 gm/kg. During the next ten days, the concentration of alcohol offered was halved, and in this case the intake also declined although proportion stayed relatively constant. However, when the 15% concentration was reinstated, the intake measure increased, and when the alcohol concentration was doubled to 30%, the g per kg measure increased even though the proportion declined.

From this result (Table 3), it is apparent that the preference and large intake of alcohol persist well beyond the discontinuation of the infusion of THP intraventricularly. Further, it would appear that a higher, rather than lower, concentration of alcohol enables the animal to sustain its high intake of alcohol in response to the dopamine metabolite.

*THP Dose Response*

A complicated dose-response relationship is evident because of (a) the potency of the alkaloid conjugate, and (b) the ostensibly differential dispersion of the metabolite, with each intraventricular infusion, from animal to animal. As noted in Fig. 3, a dose-response relationship is only obtainable if very low concentrations are used, with higher doses frequently attenuating or even blocking the alcohol-inducing action of THP.

As shown in Table 4, when the low dose of 0.2 µg was increased ten-fold, in two animals, the g per kg intakes increased substantially. However, in one animal which was already consuming 5.0 g per kg, such an increase reduced the animal's intake by one-half. This demonstrated the fact that a higher dose can counteract the metabolite's central action. This finding was also demonstrated in two additional rats (No. 35 and 36) in which the 1.0 µg dose was doubled. Again the effect was a substantial reduction in proportion and gram intake during the second alcohol preference sequence (Table 4). From these observations, the conclusion could be drawn that a dose of THP delivered centrally should be reduced by 10 or even a 100-fold should that test dose not be efficacious.

*Taste Factor and THP*

After alcohol in the 3 to 30% range of concentrations had been adulterated with $2 \times 10^{-5}$ M saccharin [15], each solution was offered simultaneously with water and the $2 \times 10^{-5}$ M saccharin solution alone, in the second and third drinking tubes. Under this three-choice condition, the THP-infused rats nevertheless consumed alcohol. As shown in Table 5, the rats did drink a saccharin solution as expected, but preferred the one which contained alcohol. In fact, the overall mean proportion and g per kg intakes of alcohol either alone or as a mixture with the saccharin solution, were virtually identical.

When a solution of Sustagen, isocaloric with either 20% or 10% alcohol was offered in the third drinking tube, along with water and alcohol in the other two tubes, the volume of fluids consumed by the THP-infused animals actually tripled. Table 6 shows that the average amount of Sustagen solution taken in the presence of 20% and 10% alcohol, each for three-day periods, was 110 and 115 ml respectively. In spite of this, however, the THP-infused rats consumed on the average 4.8 ml of 20% and 8.8 ml of 10% alcohol. When the proportion measure was calculated, the alcohol to water ratios again showed that the relative preference for alcohol was very high. In addition, the intake of alcohol ranged between 1.4 and 2.0 g per kg per day even though the rats were probably in a state of maximum

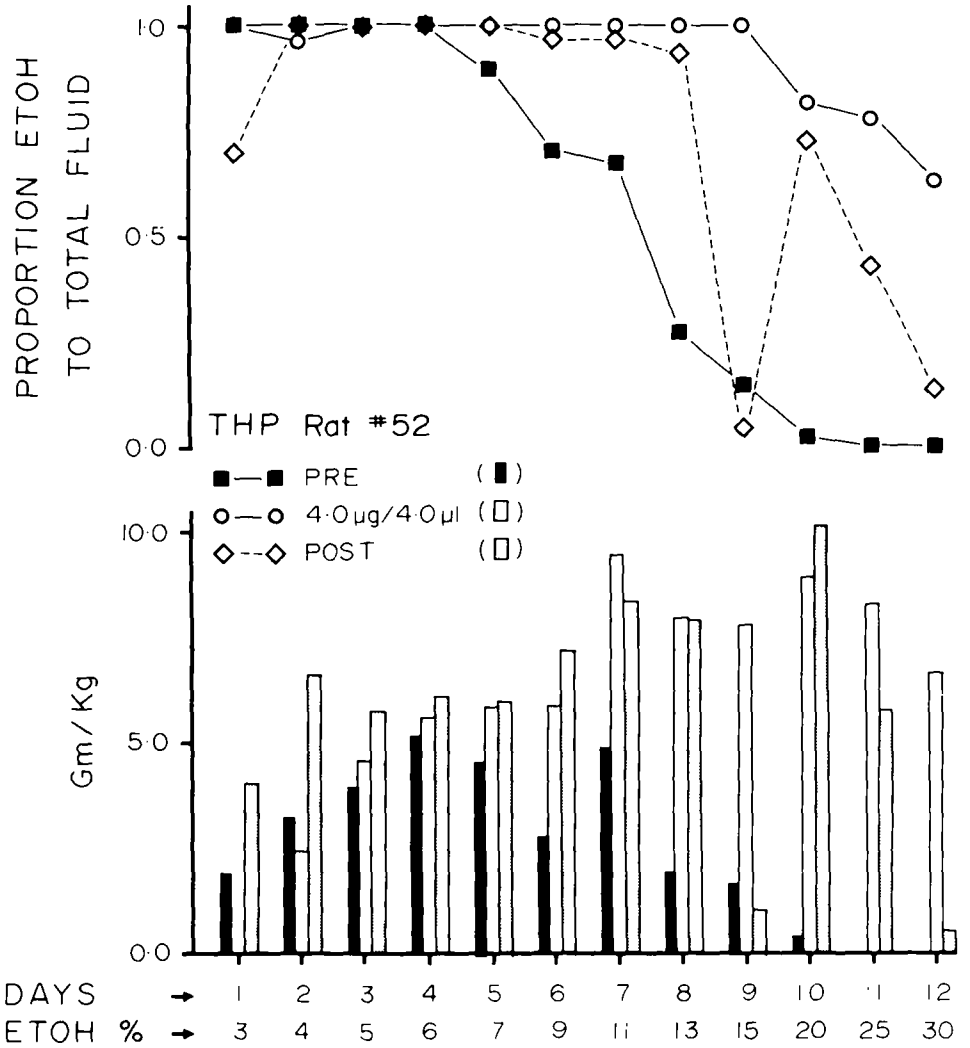


FIG. 6. The proportion of alcohol to total fluid intake (TOP) and g alcohol per kg body weight (BOTTOM) consumed for each concentration of alcohol offered to rat No. 52 during the intraventricular infusion of 4.0 μl every 30 min of 4.0 μg of THP and during the succeeding infusion of CSF started one month after the beginning of the infusion of THP.

TABLE 3

THE PROPORTION OF ALCOHOL (ETOH) TO TOTAL FLUID INTAKE AND Gm OF ALCOHOL PER Kg BODY WEIGHT CONSUMED BY TWO RATS IN WHICH 4.0 μg/4.0 μl OF THP HAD BEEN INFUSED INTRAVENTRICULARLY EVERY 30 MIN FOR 14 DAYS PRIOR TO THE PRESENTATION OF AN ALCOHOL TEST SEQUENCE (12 DAYS) AND THEN FOUR PERIODS WHEN CONSTANT CONCENTRATIONS OF ALCOHOL WERE OFFERED (15, 7.5, 15 AND 30 PERCENT)

| % ETOH Offered | Duration (days) | Rat No. 114 | | Rat No. 115 | |
|---|---|---|---|---|---|
| | | Proportion | gm/kg | Proportion | gm/kg |
| 3–30 | 12 | 0.67 | 5.4 | 0.65 | 3.2 |
| 15 | 10 | 0.60 | 6.6 | 0.66 | 6.3 |
| 7.5 | 10 | 0.54 | 3.0 | 0.61 | 2.7 |
| 15 | 14 | 0.43 | 4.1 | 0.53 | 4.8 |
| 30 | 12 | 0.27 | 5.1 | 0.29 | 6.1 |

TABLE 4

MEAN PROPORTION OF ALCOHOL (ETOH) TO TOTAL FLUID INTAKE AND MEAN Gm OF ALCOHOL PER Kg OF BODY WEIGHT INGESTED DURING TWO SUCCESSIVE ETHANOL TESTS BY RATS INFUSED INTRAVENTRICULARLY WITH DIFFERENT DOSES OF THP

| | Prescreen Control | | First ETOH Test | | | Second ETOH Test | | |
|---|---|---|---|---|---|---|---|---|
| Animal | Proportion | gm/kg | Dose ($\mu$g/$\mu$l) | Proportion | gm/kg | Dose ($\mu$g/$\mu$l) | Proportion | gm/kg |
| 40 | 0.10 | 0.1 | 0.2 | 0.44 | 2.9 | 2.0 | 0.35 | 3.6 |
| 41 | 0.28 | 0.8 | 0.2 | 0.45 | 5.0 | 2.0 | 0.45 | 2.6 |
| 42 | 0.28 | 1.6 | 0.2 | 0.70 | 3.1 | 2.0 | 0.84 | 7.0 |
| 35 | 0.04 | 0.3 | 1.0 | 0.67 | 3.2 | 2.0 | 0.52 | 2.5 |
| 36 | 0.42 | 1.7 | 1.0 | 0.83 | 4.7 | 2.0 | 0.69 | 3.6 |

TABLE 5

MEAN PROPORTION OF ALCOHOL (ETOH) TO TOTAL FLUID INTAKE AND MEAN Gm OF ALCOHOL PER Kg OF BODY WEIGHT CONSUMED BY EACH ANIMAL BEFORE INFUSION AND DURING THE INTRAVENTRICULAR INFUSION OF 2.0 $\mu$g/$\mu$l OF THP FOR BOTH AN ALCOHOL PREFERENCE TEST AND THE SACCHARIN TEST

| | Prescreen Control | | ETOH Alone | | ETOH in Presence of Saccharin | |
|---|---|---|---|---|---|---|
| Animal | Proportion | gm/kg | Proportion | gm/kg | Proportion | gm/kg |
| 30 | 0.14 | 0.6 | 0.22 | 2.9 | 0.60 | 5.6 |
| 36 | 0.42 | 1.7 | 0.69 | 3.6 | 0.47 | 4.0 |
| 40 | 0.11 | 0.1 | 0.35 | 3.6 | 0.16 | 0.5 |
| 41 | 0.28 | 0.8 | 0.45 | 2.6 | 0.54 | 3.6 |
| 42 | 0.29 | 1.6 | 0.84 | 7.0 | 0.73 | 4.7 |
| Overall Mean | 0.25 | 1.0 | 0.51 | 3.9 | 0.50 | 3.7 |

TABLE 6

THE MEAN MLS OF FLUID, PROPORTION OF ALCOHOL (ETOH) TO WATER, AND Gm OF ALCOHOL PER Kg BODY WEIGHT CONSUMED ON EACH DAY BY THREE RATS IN WHICH 4.0 $\mu$g/4.0 $\mu$l WAS INFUSED INTRAVENTRICULARLY DURING THE PRESENTATION OF A SOLUTION OF SUSTAGEN ISOCALORIC WITH THE CONCENTRATION OF ETHANOL OFFERED

| % ETOH | Days | ETOH ml | Water ml | Sustagen ml | Proportion of ETOH to ETOH plus water | gm/kg |
|---|---|---|---|---|---|---|
| | 1 | 5.7 | 0 | 123 | 1.00 | 2.0 |
| 20 | 2 | 4.0 | 0.3 | 94 | 0.93 | 1.4 |
| | 3 | 4.7 | 0.6 | 114 | 0.89 | 1.5 |
| Overall Mean | | 4.8 | 0.3 | 110 | 0.94 | 1.6 |
| | 1 | 9.7 | 3.7 | 115 | 0.72 | 1.6 |
| 10 | 2 | 9.0 | 1.0 | 124 | 0.90 | 1.6 |
| | 3 | 7.7 | 6.3 | 107 | 0.55 | 1.4 |
| Overall Mean | | 8.8 | 3.7 | 115 | 0.72 | 1.5 |

hydration because of the inordinately large intakes of Sustagen solution.

*Diurnal Alcohol Drinking Patterns*

The drinking patterns reflected by the drinkometer records revealed that the THP-infused rats consumed nearly 80% of their total daily alcohol intake during the night-time interval, i.e., their waking period. Starting at 6 to 8 p.m., the periods of drinking peaked at intervals of about two hr, the onset of which was signalled by the absence of illumination in the laboratory room. Figure 7 presents a representative pattern of alcohol drinking in a rat in which

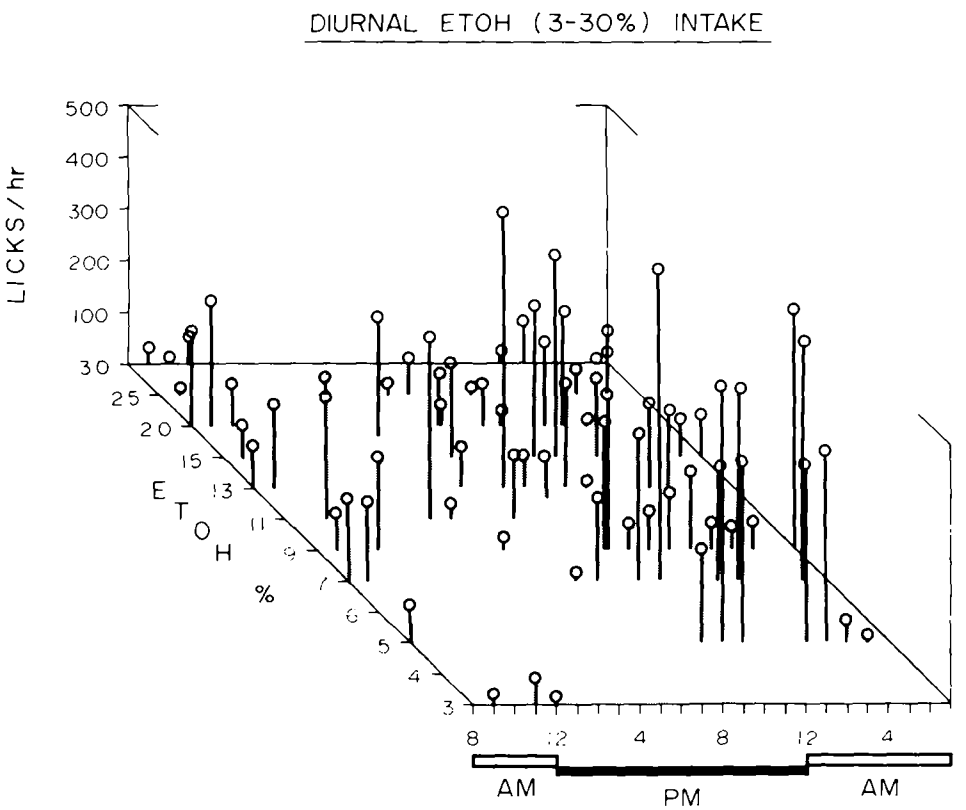


FIG. 7. Drinkometer responses for each hour during the second alcohol test sequence for one rat in which 4.0 μg/4.0 μl of THP had been infused intraventricularly every 30 min during the first alcohol test. CSF constituted the infusate at this time. The vertical axis shows the total number of licks on the tube containing alcohol for each hour; the oblique axis indicates the concentration of alcohol offered on each day; and the horizontal axis shows the time of day. The opened and closed bars below the horizontal axis indicate a.m. and p.m., respectively.

the 4.0 μg dose of THP had been infused intraventricularly every 30 min during the alcohol preference sequence. At the 5% concentration, two bouts of drinking occurred (Fig. 7), whereas at the higher concentrations the drinking of alcohol (e.g., 20%) was temporally distributed in that drinking occurred even at 8 and 9 a.m.

In two animals in which the 4.0 μg dose had produced excessive intake during the 3 to 30% preference sequence, a constant concentration was offered to each animal for 12 consecutive days. In both cases, each rat had a choice of water and a 30% concentration of alcohol. Two very different patterns of intake were observed. In one rat, the drinking pattern depicted in Fig. 8 was characterized by one or at most two intense bouts of alcohol drinking during the course of a 24-hr period. There was essentially no drinking of this 30% concentration of alcohol between the hours of 8 a.m. and 1 a.m. on the following day. A different distribution of alcohol intake was noted in the second animal as portrayed in Fig. 9. In this instance, the rat sampled the 30% concentration much more frequently, distributing its intake throughout the course of the night portion of the diurnal cycle. In fact, on some days, only 6 to 8 hr elapsed during the day portion of the cycle in which the rat failed to drink some volume of the 30% alcohol solution.

*Changes in Blood Alcohol Level*

An analysis of samples of blood collected at different intervals during the course of 24 hr in different animals is presented in Table 7. As would be anticipated, the highest levels of alcohol in the blood were noted in samples taken immediately after a peak period of alcohol drinking. Typically, this occurred during the middle of the night-time interval. In agreement with previous reports [28], when the blood alcohol of the rat reaches approximately 0.1%, intoxication-like symptoms, such as ataxia, are clearly evident. Generally speaking, however, even in those animals that consumed large amounts of alcohol, the blood alcohol concentration was not greatly elevated at the 8 a.m. reading in spite of the fact that high volumes had been consumed during the night-time interval. Because of the rapid rate of the metabolism of alcohol and the subsequent clearance of the fluid, this finding in the rat was not unexpected.

At the peak of the night-time drinking period, those THP-infused rats maintained on a 20% concentration showed blood alcohol levels ranging from 0.62 to 0.99%. Their total intake over the entire day ranged between 4.7 and 9.5 g per kg.

*Withdrawal-Like Symptoms and Food Intake*

In seven of the animals in which THP had been infused intraventricularly, audiogenic seizures and running episodes were readily induced by simply exposing the animal to the jingling of keys or an alarm bell. One of these animals also exhibited spontaneous convulsions without sound induction. Although audiogenic seizures could be elicited as early

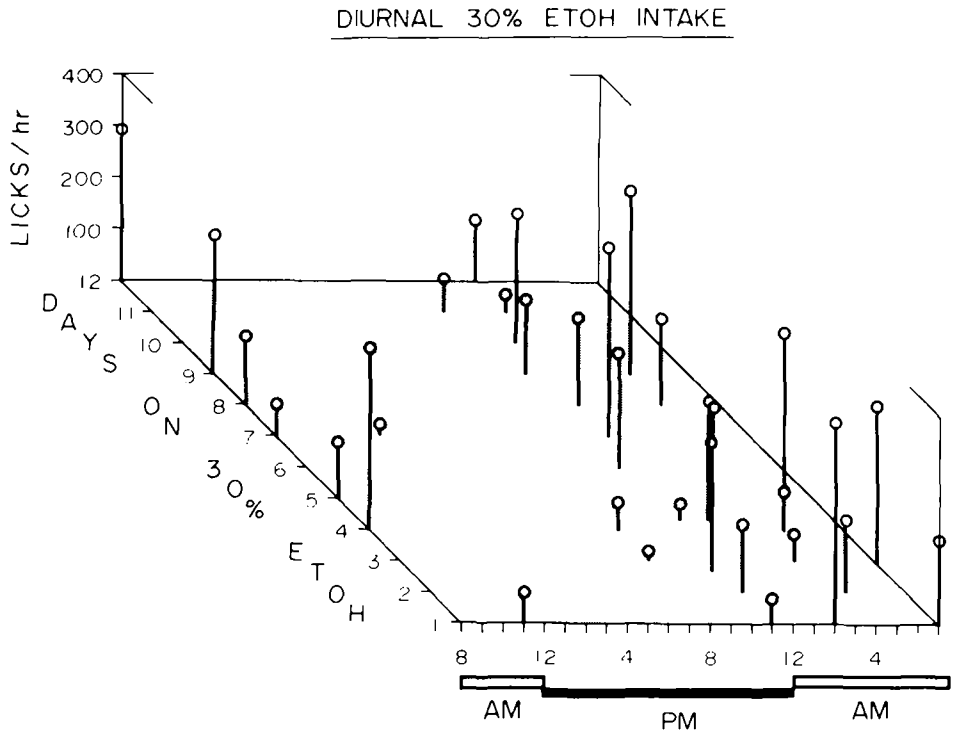


FIG. 8. Drinkometer responses for each hour during the presentation of 30 percent alcohol to a rat in which 4.0 μg/4.0 μl of THP had been infused intraventricularly every 30 min for 14 days. The vertical axis shows the total number of licks on the tube containing alcohol for each hour; the oblique axis indicates the number of days 30 percent alcohol was offered; and the horizontal axis shows the time of day. The opened and closed bars below the horizontal axis indicate a.m. and p.m., respectively.

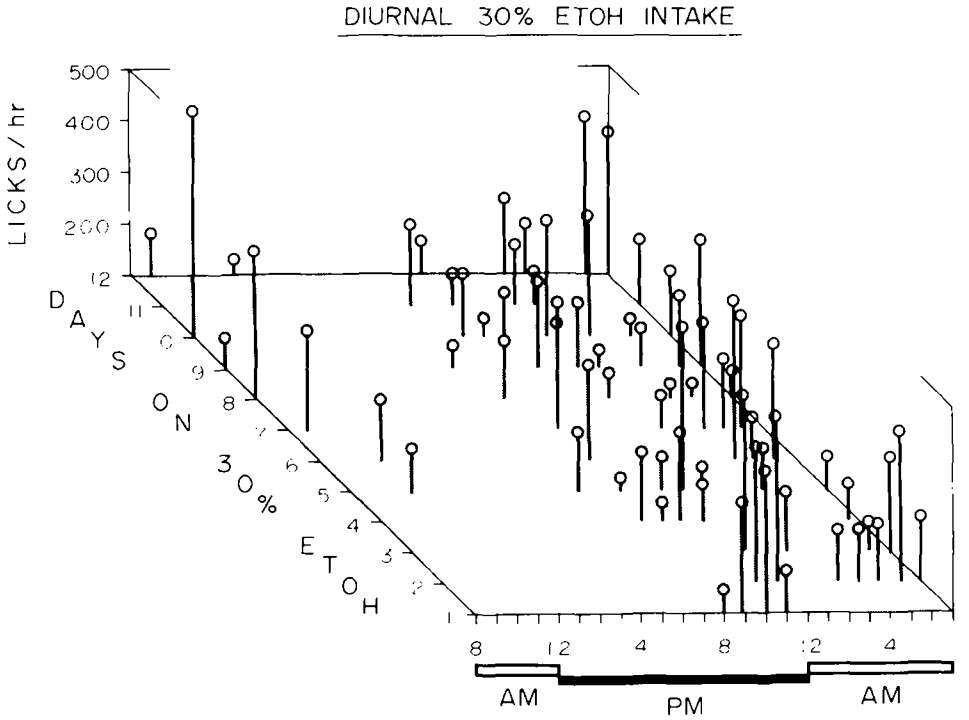


FIG. 9. Drinkometer responses for each hour during the presentation of 30 percent alcohol to a rat in which 4.0 g/4.0 μl of THP had been infused intraventricularly every 30 min for 14 days. The vertical axis shows the total number of licks on the tube containing alcohol for each hour; the oblique axis indicates the number of days 30 percent alcohol was offered; and the horizontal axis shows the time of day. The opened and closed bars below the horizontal axis indicate a.m. and p.m., respectively.

TABLE 7

PERCENT OF ALCOHOL IN THE BLOOD (BAL) OF ANIMALS INFUSED WITH 0.02 μg/μl OF THP AT 8 A.M. AND 8 P.M. DURING THE PRESENTATION OF THE ALCOHOL SOLUTIONS OF THE LAST HALF OF THE TEST SEQUENCE. ALSO, THE BAL OF 3 RATS INFUSED WITH 4.0 μg/4.0 μl OF THP AT PEAK NIGHT TIME DRINKING PERIODS DURING THE PRESENTATION OF A CONSTANT CONCENTRATION OF ALCOHOL, 20%. THE g/Kg MEASURE IS FOR 24 HR EXCEPT FOR THE 8 P.M. READINGS IN WHICH IT INDICATES THE 12 HR INTAKE

| 8 a.m. | 11% ETOH | | 13% ETOH | | 15% ETOH | | 20% ETOH | | 25% ETOH | | 30% ETOH | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Animal | BAL | g/kg | BAL | g/kg | BAL | g/kg | BAL | g/kg | BAL | g/kg | BAL | g/kg |
| 45 | .003 | 7.1 | .000 | 7.7 | .052 | 6.9 | .041 | 9.2 | .043 | 10.0 | .008 | 7.4 |
| 46 | .023 | 11.6 | .009 | 4.0 | .037 | 4.6 | .007 | 11.0 | .086 | 7.8 | .033 | 5.8 |
| 47 | .000 | 4.7 | .040 | 11.2 | .008 | 2.3 | .000 | 10.8 | .057 | 9.6 | .034 | 3.4 |
| 48 | .000 | 9.0 | .032 | 7.3 | .000 | 8.4 | .037 | 10.3 | .023 | 9.7 | .005 | 7.7 |
| 49 | .017 | 7.4 | .000 | 10.6 | .009 | 6.4 | .040 | 11.2 | .040 | 10.6 | .007 | 10.1 |

| 8 p.m. | 25% ETOH | | 30% ETOH | |
|---|---|---|---|---|
| Animal | BAL | g/kg | BAL | g/kg |
| 45 | .206 | 2.9 | .198 | 2.3 |
| 46 | .031 | 3.6 | .104 | 1.5 |
| 47 | .000 | 3.4 | .040 | 1.4 |
| 48 | .046 | 5.2 | .039 | 3.1 |
| 49 | .082 | 5.6 | .079 | 5.4 |

| Peak Night Drinking | 20% ETOH | | 20% ETOH | |
|---|---|---|---|---|
| Animal | BAL | g/kg | BAL | g/kg |
| 50 | .099 | 7.1 | .062 | 7.1 |
| 52 | .087 | 8.0 | .087 | 9.5 |
| 54 | .079 | 4.7 | .079 | 7.7 |

TABLE 8

THE AVERAGE DAILY FOOD INTAKE AND g OF ALCOHOL PER Kg OF BODY WEIGHT CONSUMED DURING AN ALCOHOL PREFERENCE TEST BY ANIMALS IN WHICH CSF OR 2.0 OR 0.02 μg/μl OF THP WAS INFUSED INTRAVENTRICULARLY. RESULTS ARE PRESENTED FOR THE FIRST HALF (3–9%) AND SECOND HALF (11–30%) AS WELL AS THE ENTIRE (3–30%) TEST SEQUENCE

| Dose of THP | | Food (g) | | | (g/kg) | | |
|---|---|---|---|---|---|---|---|
| g/μl | Animal | 3–9% | 11–30% | 3–30% | 3–9% | 11–30% | 3–30% |
| 2.0 | 6 | 15.6 | 19.3 | 17.5 | 2.8 | 6.3 | 4.5 |
| | 9 | 9.8 | 14.7 | 12.3 | 2.3 | 4.2 | 3.2 |
| | 25 | 27.8 | 30.2 | 29.0 | 6.6 | 8.3 | 7.4 |
| | 26 | 27.7 | 37.0 | 32.3 | 1.9 | 3.7 | 2.8 |
| | 29 | 19.8 | 28.2 | 24.0 | 3.8 | 2.3 | 3.1 |
| | Mean | 20.1±7.8 | 25.9±8.9 | 23.0±8.2 | 3.4±1.9 | 4.9±2.4 | 4.2±1.9 |
| 0.2 | 45 | 22.2 | 23.3 | 22.8 | 5.0 | 8.1 | 6.6 |
| | 46 | 16.8 | 17.0 | 16.9 | 5.6 | 7.5 | 6.6 |
| | 48 | 14.7 | 12.8 | 13.8 | 3.8 | 8.7 | 6.3 |
| | 49 | 34.8 | 39.5 | 37.2 | 5.0 | 9.4 | 8.0 |
| | Mean | 22.1±9.0 | 23.2±11.8 | 22.7±10.4 | 4.9±0.8 | 8.4±0.8 | 6.6±1.0 |
| CSF | 2 | 17.3 | 39.3 | 26.1 | 1.0 | 1.1 | 1.0 |
| Control | 17 | 22.7 | 36.8 | 29.1 | 0.2 | 0.4 | 0.3 |
| | 31 | 24.4 | 35.3 | 29.2 | 0.0 | 0.1 | 0.3 |
| | 33 | 19.0 | 37.8 | 27.5 | 0.1 | 1.8 | 1.0 |
| | Mean | 20.9±3.3 | 37.3±1.7 | 28.0±1.5 | 0.3±0.5 | 0.9±0.8 | 0.6±0.4 |

as the fourth day of a THP infusion sequence, in most instances seizure episodes did not develop until at least 8 days after the infusion series began.

Many animals exhibited symptoms such as teeth chattering, stiffness of the tail, wet dog shakes and rather marked tremors of the head and other areas of the body. Frequently, signs of hyperactivity were noted with many animals actually dislodging their food cups and overturning them. Ordinarily, the withdrawal-like symptoms again were not observed until the third or fourth day following the initiation of chronic infusions of THP. Once the signs appeared, they usually persisted for the remainder of the

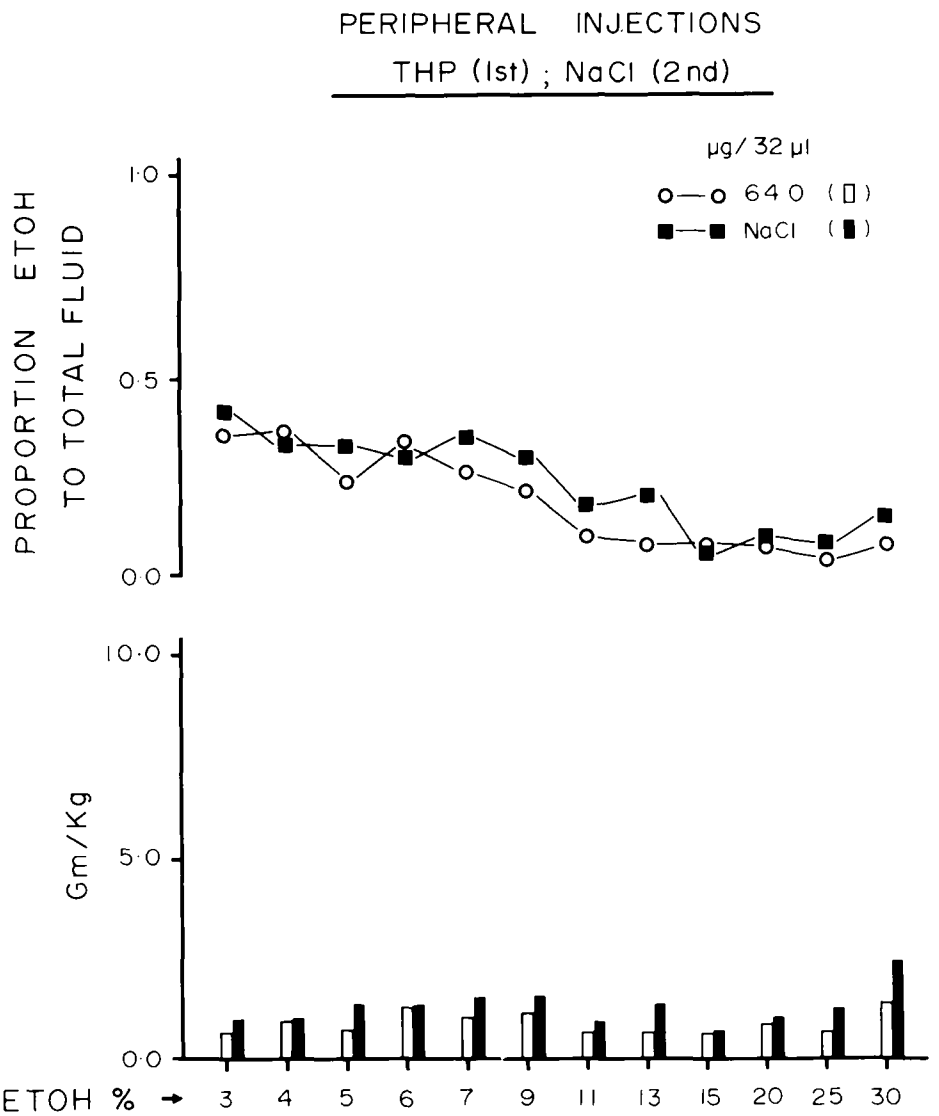


FIG. 10. The proportion of alcohol to total fluid intake (TOP) and g of alcohol per kg body weight (BOTTOM) for the rats intraperitoneally injected three times per day with 32.0 µl containing 64.0 µg of THP during their first alcohol test sequence and 32.0 µl of saline during their second test sequence.

sequence. In animals exhibiting the more severe behavioral changes, the less severe symptoms of withdrawal were also present.

In those rats in which 2.0 or 0.02 µg of THP were infused intraventricularly, the intake of food during the alcohol preference sequence tended to be somewhat lower. As presented in Table 8, the mean food intake of the control rats was reduced during the 6 days when the 3 to 9% concentrations of alcohol were available. During the latter half of the sequence, 11–30% alcohol concentrations, the food intake averaged 37 g per day. This could reflect the fact that the animals derived a portion of their calories from alcohol and consumed, therefore, correspondingly less food during the first half of the preference sequence.

Overall, there were no statistically significant differences between the groups in terms of food intake throughout the course of the infusion sequences. Polydipsia and polyurea were noted in six animals, in which THP was infused intraventricularly, but these symptoms were independent of the dose of the alkaloid metabolite given. Within two to four days of the beginning of infusion, the rats frequently consumed over 90 ml of fluid per day. In two animals, the g per kg intake of alcohol was less than 0.5 g. Because of the aberrant nature of the drinking, the polydipsic rats were excluded from the analysis of data of alcohol consumption.

*Peripheral and Acute Injections*

In the two groups of six rats in which a dose of 64 µg of THP was given three times a day, no change in alcohol preference occurred in terms of the proportion value or in the actual intake of the fluid. Figure 10 presents the pattern of drinking for one group in which the THP was given during the first sequence and the saline control vehicle during the second. Table 9 presents a comparison of the proportion and g per kg intakes for both groups given intraperitoneal injections of either the saline vehicle or THP. The very slight rise in proportion and g intake could be attributed to the acclimation effect that arises upon

TABLE 9

A COMPARISON OF THE PROPORTION OF ALCOHOL TO TOTAL FLUID INTAKE AND THE g/Kg OF ALCOHOL CONSUMED DURING ALCOHOL SEQUENCES FOR THE RATS RECEIVING INTRAPERITONEAL INJECTIONS OF SALINE OR 64 μg OF THP THREE TIMES A DAY. N = 6 FOR BOTH GROUPS

| Group | Sequence | Treatment | Proportion | g/kg |
|---|---|---|---|---|
| A | 1 | Saline | 0.10 ± 0.04 | 0.43 ± 0.22 |
| (N = 6) | 2 | THP | 0.16 ± 0.11 | 0.73 ± 0.40 |
| B | 1 | THP | 0.18 ± 0.09 | 0.89 ± 0.48 |
| (N = 6) | 2 | Saline | 0.23 ± 0.12 | 1.31 ± 0.64 |

TABLE 10

PROPORTION OF ALCOHOL TO TOTAL FLUID INTAKE AND g OF ALCOHOL PER Kg OF BODY WEIGHT CONSUMED BY ANIMALS IN WHICH 20.0 μl OF 2.0 μg/μl OF THP WAS INJECTED ACUTELY. THE RESULTS ARE EXPRESSED IN TERMS OF FIRST HALF (3–9%) AND SECOND HALF (11–30%), AS WELL AS THE ENTIRE 3–30% ALCOHOL TEST SEQUENCE

| | Prescreen Control | | | | | | Post THP Injection | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Proportion | | | g/kg | | | Proportion | | | g/kg | | |
| Animal | 3–9% | 11–30% | 3–30% | 3–9% | 11–30% | 3–30% | 3–9% | 11–30% | 3–30% | 3–9% | 11–30% | 3–30% |
| 80 | 0.83 | 0.14 | 0.50 | 2.3 | 1.6 | 1.9 | 0.71 | 0.46 | 0.58 | 2.7 | 4.2 | 3.4 |
| 220 | 0.12 | 0.34 | 0.53 | 2.3 | 3.3 | 2.8 | 0.94 | 0.54 | 0.74 | 2.2 | 3.4 | 2.8 |
| 211 | 0.16 | 0.03 | 0.10 | 0.4 | 0.5 | 0.5 | 0.55 | 0.16 | 0.36 | 1.9 | 2.3 | 2.1 |
| 216 | 0.04 | 0.04 | 0.04 | 0.1 | 0.4 | 0.2 | 0.04 | 0.05 | 0.05 | 0.1 | 0.4 | 0.2 |
| 132 | 0.12 | 0.01 | 0.07 | 0.3 | 0.1 | 0.2 | 0.18 | 0.12 | 0.15 | 0.2 | 0.7 | 0.4 |

repeated exposure to alcohol [37].

A single intraventricular injection of a high dose of 40 μg in 20 μl of vehicle exerted a variable effect in the five animals tested, as indicated in Table 10. With but one exception there were virtually no significant changes in alcohol drinking. In animal No. 211, the proportion value and g per kg intakes increased by as much as three-to-five-fold. These results indicate that an element of chronicity is required in the induction of drinking by the amine condensation product acting within the brain.

## DISCUSSION

Several striking characteristics of the central action of THP, infused chronically into the brain of the rat, are readily apparent. First, THP induces excessive imbibition of alcohol in a volume which has not been reported previously [10, 29, 37]. Second, when very high concentrations of alcohol are offered in the gustatorily aversive range, the rat nevertheless selects alcohol in preference to water in the free-choice situation. Third, long after the cessation of the series of chronic infusions of THP, the rat continues to drink substantial quantities of alcohol when the fluid is once again made available in the free-choice situation with water. Fourth, withdrawal-like symptoms similar to those reported for an animal made artificially dependent upon alcohol (e.g., [28]) are also produced. Fifth, only trace amounts of THP are required in the brain to evoke these remarkable functional changes.

Overall therefore, this profile of responses seems to simulate a clinical condition which would be analogous to the alcoholic patient: immoderate drinking voluntarily, the ingestion of alcohol even under aversive conditions, the persistence of the drinking pattern after a protracted period of abstinence, and symptoms of withdrawal. But for what reason does the rat drink alcohol in response to THP? Why do progressive increases in alcohol consumption, occurring during the first half of the 12-day alcohol preference test, continue to the point which may exceed even the maximal level of metabolism [22] of alcohol? There are several alternatives.

One possibility is that THP impairs a rat's capacity to discriminate the taste of the fluids. However, one would predict that a proportion measure of 0.50 would characterize the drinking pattern of the rat across the 12 concentrations of alcohol offered simultaneously with water. This did not occur. In addition, several rats selected unusual quantities of alcohol even when a more palatable solution was made available. Moreover, intraventricular injections of THP on successive days fail to alter the acceptance-rejection curves for 12 different concentrations of quinine solution [36]. In this connection, it is significant that the rat undergoing withdrawal from morphine exhibits an increased intake of alcohol in terms of its preference for an alcohol-sucrose solution rather than a sucrose solution alone [16]; again, these self-same animals exhibited no difference from the controls in their preference-aversion to quinine [16].

Although the mechanism for the persistence of the exaggerated preference long after the THP infusions have ceased is presently unknown, it would appear likely that this condensation product induces an alteration in neuronal metabolism, protein structure or even a morphologically specific ultrastructural lesion within the rat's limbic system. Of course, it is possible that THP itself is still present in the brain parenchyme; but this is unlikely since enzymes are present which would cause its degradation [4]. Alternatively, THP may react with the sulfhydryl groups of proteins [43] which could disturb normal cellular functions

by altering, for example, the binding site for membrane calcium [42].

With respect to a local chemical lesion to a structure lining the wall of the rat's ventricle, 6-OHDA and 5, 6-DHT do exert an effect on a rat's preference for alcohol [15, 27, 34]. Further, 6-OHDA influences tolerance but not dependence [40]. However, neither the catecholaminergic nor serotonergic neurotoxin induces a shift in alcohol preference which in any way compares with that seen in the present investigation.

One might expect that a progressive synergism between THP and alcohol arises as the animal drinks the fluid at the same time that the brain is exposed to the condensation product. This possibility is not borne out because rats offered alcohol after the completion of a long period of THP infusion exhibit the same intense preference for the fluid. This indicates that a pairing of THP acting centrally with alcohol, as derived systemically, is not necessary.

The fact that THP administered peripherally exerts no effect on alcohol drinking, in doses efficacious centrally, unequivocally pinpoints the action of the alkaloid conjugate to the central nervous system. The difficulty in generating a THP dose-response relationship, particularly at the lower end of the dose range, underscores the fact that only minute amounts of THP are critical. One might predict, therefore, that a TIQ could not be detected readily in the brain of an animal following its ingestion of alcohol [38], unless the biosynthesis of a given TIQ is enhanced or its metabolic degradation inhibited by a pharmacological agent [5,46]. Indeed, the lower limit of sensitivity of the assays employed is reportedly in the range of 5 to 10 nanograms per g of brain tissue. If one considers that the lowest effective dose of THP is 0.4 ng per infusion and that the microliter volume is subjected to an enormous dilution [24] prior to its reaching the site of action, it is readily understandable that the assay systems currently in use will not detect and identify a TIQ, at least at the level that would induce a physiological effect [6].

The occurrence of withdrawal-like activity in the rat in which THP is infused into its brain is not surprising. Another in the family of TIQ compounds, 6,7-dihydroxy-TIQ, exacerbates withdrawal symptoms in the mouse for a period of 20 hr following a single intraventricular injection [2]. Just as in our study, the morphological site of action has not as yet been delineated. Neither THP nor 6,7-dihydroxy-TIQ microinjected into the nucleus accumbens of the rat elicits hyperactivity although other TIQs applied similarly to this area do evoke locomotor activity for an extended period [7]. The lack of information about the locus of THP's action within the CNS is not exclusive to the TIQ, since the region in which naloxone exerts its effect is equally ill-defined even though this problem has been investigated extensively [47,48]. The most severe symptom of withdrawal, a convulsive episode, occurs usually following the disappearance of alcohol from the blood [23,25]. Nevertheless, as seen in the present investigation, a seizure may develop during a period when the animal ingests alcohol and has a level of alcohol in the blood approaching 0.1% [23]. Of interest is the fact that a blood alcohol level, similar to that in the animal infused with THP, has been reported to produce signs of physical dependence in the rat [23].

In conclusion, the field of experimental alcoholism has long been beleaguered by the lack of a valid animal model [29]. Since the component of voluntary selection of alcohol is indeed the critical factor in the development of such a model [10, 30, 37], the results of the current investigation may provide the basis for future experimental studies of this drinking phenomenon. In any event, a tetrahydroisoquinoline or one of its derivatives, when acting within the central nervous system, would certainly seem to be involved in the etiology of the pathological drinking of alcohol.

ACKNOWLEDGEMENTS

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