Alcohol Consumption in Rats: Effects of Intracranial Injections of Ethanol

Abstract. *After permanent implantation of intraventricular cannulae, rats were repeatedly infused with ethanol solutions of several concentrations. Pronounced preferences were exhibited for ordinarily noxious ethanol without prior oral exposure to this fluid. Since preferences for ethanol remained unusually high after cessation of infusion, a permanent change may take place in the central nervous system.*

Most strains of rats will not ordinarily drink ethanol except in solutions of very low concentration. However, under special circumstances, such as stress (*1*) or habituation (*2*), a solution of ethanol may become a fluid of choice if a sufficient acclimation period is permitted. Because of this temporal requirement, a central nervous factor may be related to the imbibition of this gustatorily noxious substance.

The role of the central nervous system in human alcoholism as a disease is poorly understood, but there is much evidence that the state of the nervous system is significantly related to one's selection of alcohol. In addition, it is possible that a tissue or cellular change of a biochemical nature occurs with continued alcohol imbibition. Such a change in the nervous system then could sustain the chronic alcoholic's intake once the alteration has taken place.

To partially test this hypothesis, a new technique was designed (*3*) whereby ethanol could be infused intracranially for prolonged periods of simulated chronicity. The ultimate preference threshold for oral ethanol solutions was then investigated as a function of the amount, concentration, duration, and schedule of intracranially infused ethanol.

Five groups of from two to four adult male rats of the Colgate strain, never previously exposed to ethanol, were stereotaxically implanted with unilateral 23-gauge syringe needle cannulae, so that the tips of the cannulae rested in cerebrospinal fluid (*4*). Intracranial infusion was then initiated and continued by means of programmed infusion pumps which delivered fluid to the cannulae through PE-10 tubing according to a procedure described previously (*3*). Schedules were maintained in which 1 to 3 μl of 5- and 10-percent ethanol (*5*) were administered at a rate of one infusion every 15 minutes throughout a 10-day period, so that each rat received 1000 intraventricular microinjections in all. Control rats were infused with isotonic saline. At the end of the 10-day period, all rats were deprived of food and water, then preference tested with ethanol and water, according to the tri-operant procedure of Myers and Carey (*6*) for food and fluid. The concentrations of the solutions were increased daily from 3 to 10 percent, and were extended when oral preferences persisted beyond the 10-percent concentration. The positions of the fluids were exchanged daily, according to the standard techniques for measuring preference.

During the daily tests, uncommon quantities of ethanol were consumed by rats in the experimental group. Figure 1 presents an analysis of the relationships between the amount of the intracranial ethanol dosage and the ultimate oral concentration preferred in the self-selection situation. The group infused with 1 μl of a 5-percent solution drank up to 8 percent ethanol (ETOH), whereas those groups infused with 1 μl of a 10-percent, or 2 μl of a 5-percent solution (actually equilibrated amounts) maintained oral preference for solutions up to 10 percent. All ethanol offerings through 30 percent were chosen by the group which received the highest dosage of 3 μl of 10-percent ethanol. The responses of the controls were similar to those of rats of the same strain which had not been operated on (*7*). Differences between groups were significant from one another (all t-tests were greater than 2.98, $p = <.05$) except between the controls and the group that received 1 μl of 5-percent ethanol ($t = .94$) and between the groups that received 1 μl of 10-percent and 2 μl of 5-percent ethanol ($t = 1.11$).

Since intracranial infusion for 10 days was sufficient to evoke strong ethanol preference, the question arose as to the latency of the preference shift following initiation of infusion. Secondly, would the effects of infusion be lasting if the choice between ethanol and water was offered subsequent to the discontinuation of infusion.

A new self-selection procedure was adopted (*8*) so that preference thresholds could be ascertained without having to remove the animals from their living chambers where they received their intracranial infusions. After ventricular implantation of unilateral cannulae, six rats previously unexposed to

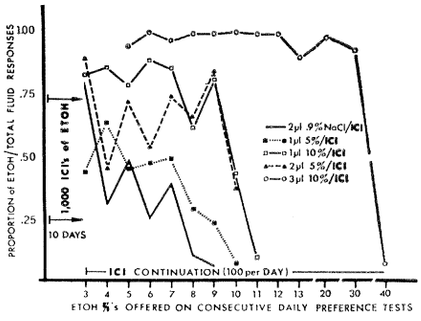

Fig. 1. Oral preferences for increasing ethanol (ETOH) concentrations during daily tests following chronic intracranial infusion (ICI).

ethanol were deprived of fluid and subjected to a "moderate" schedule in which 2 μl of 10-percent ethanol was infused every 15 minutes. Water and increasing concentrations of ethanol were offered simultaneously during each test session until preference for water rather than ethanol was unequivocally manifest. Throughout the 5 days of infusion and the 5 days following cessation of infusion, preference thresholds were determined. After 5 days of being offered as much water as desired, the animals were retested for an additional 5 days, which were followed by systematic administration of meprobamate and hydroxyphenamate (*9*) to ascertain whether ataractic drug treatment would reduce the preference.

The time course of ethanol preference following the chronic introduction of this substance into the brain is depicted in Fig. 2. Within 12 hours, a mean concentration was selected which was higher than that (6 percent) described for rats of the same strain whose

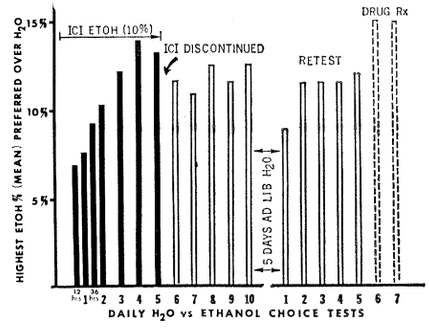

Fig. 2. Preference for ethanol as opposed to water in successive tests when the rats were receiving chronic intracranial infusions of ethanol and after infusion had ceased. After the initiation of intracranial infusion, measurements were taken at 12-hour intervals for the first 2 days, then once a day, as indicated on the abcissa.

oral fluid intakes were restricted to 20-percent ethanol for 30 days (*6*). By the fourth day of infusion 14 percent was preferred and after cessation of infusion, preference for high concentrations persisted. Moreover, neither the offering of water for a 5-day period nor the drug injections altered the oral thresholds observed during final testing. In the latter case, both drugs exhibited identical effects; they may have ameliorated slightly the noxious taste of 15-percent ethanol.

In this study, preference for ethanol (*10*) was positively related to the amount of ethanol injected intracranially over a period of time. Although rats of the strain we used normally reject even weak solutions of ethanol (*7*), the infusion of this fluid into brain tissue transformed the predisposition toward ethanol avoidance into preferences for much higher concentrations than have been reported in the literature. In addition, the persistence of the preference for over 2 weeks after cessation of infusion tends to support the hypothesis that the alteration to the central nervous sysem (*11*) may be permanent.

Although it is presently difficult to delimit the general biochemical and neural mechanisms related to fluid selection in this study, several important factors stand out in relation to the preference shifts. From autoradiographic and other evidence (*8, 12*), the improbability of systemic dispersion of ethanol infused intracranially may rule out the etiological involvement in alcoholism of the liver (*13*), endocrine glands (*14*), or bodily nutritional balance (*15*). Corroboration of this concept may rest in the comparison between rats infused intracranially and rats of the same strain orally acclimated to ethanol (*6*). The preference threshold for rats orally acclimated was 6 percent after 600 ml of 20-percent ethanol was consumed over 30 days. This is in sharp contrast to the rapid and almost linear rise in ethanol preference following the brief span of infusion (Fig. 2) of a fraction of the oral amount. For example, after infusing during a 12-hour period a total of 0.1 ml of 10-percent ethanol, equivalent to 0.000083 of the oral intake, a 7-percent solution was selected; after 48 hours or 0.00033 of the oral amount, an 11 percent solution was preferred. Essentially, the presence of ethanol in the central nervous system may have elicited greater perference for the fluid than oral acclimation, because orally ingested quantities of ethanol undergo systemic dilution and partial metabolism prior to passage to the brain.

It is also possible that intracranial infusion acts as a nonspecific stressor, causing the rats to drink ethanol to relieve the stress. However, it has been demonstrated (*7*) that an organism requires time to learn ethanol's placating qualities before preferring this fluid. The rats in this study preferred to drink the fluid without any prior experience.

Finally, the role of acetaldehyde and other metabolic intermediaries in chronic alcoholism is presently unclear. Since evidence on the degradation of alcohol by brain tissue is seemingly not incontrovertible (*13, 16*), it is possible that some of the effects of infusion are due to acetaldehyde or another derivative. In any event the results clearly indicate that chronic and direct alteration of the brain's biochemical "environment" can produce significant changes in later behavior. In this instance, a new biochemical theory of alcoholism may have to be evolved with its primary focus on a metabolic aberration of the central nervous system (*17*).

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References and Notes

1. A. Casey, *Quart. J. Studies Alc.* **21**, 208 (1960); R. Clark and E. Polish, *Science* **132**, 223 (1960); R. D. Myers, *Psychol. Repts.* **8**, 385 (1961).
2. C. P. Richter and K. Campbell, *Science* **91**, 507 (1940); R. D. Myers, *Am. Psychol.* **15**, 600 (1960).
3. R. D. Myers, *J. Appl. Physiol.* **18**, 221 (1963).
4. The Degroot coordinates used for cannula placement were: anterior, 5.8; lateral, 1.5; horizontal, + 3.0.
5. Sterile ethanol solutions for intracranial injections were volumetrically prepared with U.S.P. 95 percent ethyl alcohol and distilled H_2O. Oral solutions were prepared similarly except that tap water constituted the solvent.
6. R. D. Myers and R. Carey, *Science* **134**, 469 (1961).
7. R. D. Myers, *J. Comp. Physiol. Psychol.* **54**, 510 (1961).
8. ———, in *Thirst in the Regulation of Body Water*, M. Wayner, Ed. (Pergamon, New York, in press).
9. Dosages of 80 mg/kg of both drugs were prepared in a gum acacia suspension and injected intraperitoneally 30 minutes prior to each test session. Half of the group received one drug before one test session and the other drug before the other session. The remaining animals were given the two drugs in reverse order.
10. A table indicating mean grams of ethanol ingested at each level of oral concentration is available from me upon request.
11. Under light microscopy, analyses of coronal brain sections stained with thionin failed to reveal any evidence of morphologic changes or lesions other than those of the ventricular cannula tracts.
12. R. Rech and E. F. Domino, *Arch. Intern. Pharmacodyn.* **121**, 429 (1959); T. Harrison, *Endocrinology* **68**, 466 (1961).
13. W. Westerfeld and M. Schulman, *Quart. J. Studies Alc.* **20**, 439 (1959).
14. M. Zarrow, H. Aduss, M. Denison, *ibid.* **21**, 400 (1960).
15. R. J. Williams, *Alcoholism: The Nutritional Approach* (Univ. of Texas Press, Austin, 1959).
16. G. Bartlett and H. Barnett, *Quart. J. Studies Alc.* **10**, 381 (1949); T. Burbridge, V. Sutherland, C. Hine, A. Simon, *J. Pharmacol. Exptl. Therap.* **126**, 70 (1959).
17. Supported by National Science Foundation grants G16187 and G19843 and by grants from the Wallace Laboratories and the Colgate Research Council. I am indebted to Professor Raymond Myers for examining the brains histologically and to L. Beideman, R. Carey, B. Holman, P. Kinkaide, L. Pellegrino and W. Rogers for helping with the intracranial infusions.

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Ultramicrotome: A Simple, Easily Constructed Instrument

Abstract. *A practical microtome may be built with nominal skill, hand tools, and a drill press. Thermal specimen advance and cutting action by rod flexion eliminate pivots and critical moving parts. The cost of materials is negligible. Resultant sections are adequate for routine work in electron microscopy.*

The ultramicrotome described consists of a seasoned hardwood base which supports a flexible rod of metal. A specimen-holding chuck is fastened to the free end of the rod, which is mounted horizontally. There is a movable stage designed to hold a glass knife. A vertical guidepost set in the wooden base completes the assembly. The exploded diagram (Fig. 1) illustrates the separate parts, specifications for which are given in the legend. Substitute materials with similar physical characteristics should also be adequate. However, attention should be given to a few critical points. The grain of the wooden base should run parallel to the long axis of the instrument. This is because its coefficient of expansion is very low compared to that of cross-grain construction or other materials [increase in length per unit length per degree Celsius $\times 10^{-6}$: oak parallel to fiber, 4.92; oak across fiber 54.4; commercial aluminum, 24 to 28.7, and so forth (*1*)]. The brass rod must be very securely fastened to the back plate, preferably by soldering. It should be mounted so that it presses gently against the Teflon sleeve of the guide post throughout the entire vertical traverse of the downward cutting movement. When the rod is in its resting position the cut surface of the specimen in the chuck should be about ½