Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 3543–3546, June 1984
Medical Sciences

Toward an analogue of alcoholism in mice: Scale factors in the model

(metabolism/toxicity/mathematical model)

VINCENT P. DOLE AND R. THOMAS GENTRY

The Rockefeller University, New York, NY 10021

Contributed by Vincent P. Dole, February 21, 1984

ABSTRACT **Mice of the C57BL strain, given continuous access to 10% alcohol and plain water, with unlimited food and no stress, frequently drink enough alcohol to produce intoxicating levels in the blood. Nevertheless, this behavior does not appear to replicate the essential features of human alcoholism since the drinking lacks serious toxic effects and the intoxication occurs only as transient episodes in association with homeostatic consumption of fluid and food. It is suggested that continuous monitoring of intake and estimation of the concentration of alcohol in blood, which are now technically feasible, will permit distinction between alcoholic-type drinking and a simple licking for the flavor of alcohol in beverage concentration.**

Consumption of alcohol by human alcoholics is both voluntary and excessive. Despite suffering from gastritis, loss of social status from repeated episodes of intoxication, and progressive deterioration of health, a severe alcoholic is likely to drink compulsively until death. The question that has occupied us and many other investigators is whether study of animals under laboratory control might shed some light on this self-destructive behavior. To date no complete counterpart of human alcoholism—life-threatening toxicity from voluntary intake—has been found in animals, although many partial models of the disease are available (1–16).

The hope of understanding the basic cause of alcoholism through study of animals could be illusory since human motivation involves cognitive stresses that are not known to occur in animals. If such stress is the essential determinant of alcoholism, then animal studies will always be peripheral to the main problem, limited to analyzing the consequences rather than the causes of excessive drinking. On the other hand, this view may be too pessimistic. Some animals, such as the mice used in the present study, choose to drink solutions of alcohol in preference to plain water. Animals under constraints that force them to greater intakes of alcohol than they would exhibit if an unlimited supply of water and food were available show some of the toxic effects seen in alcoholics, but what is not known is whether these animals drink alcohol for its pharmacological effect or as a source of calories (17).

The toxic effects of alcohol depend on its concentration in vulnerable tissues and on accumulation of toxic metabolites. These variables, in turn, involve a number of scale factors, such as rates of ingestion, distribution, and elimination. The differences in scale between species are substantial, and no single scale factor adequately describes the differences. The mass of a mouse is only 0.0004 times that of a man. Although the water content per unit body weight (and therefore the volume of distribution for alcohol) is about the same in the two species, the metabolism of alcohol per unit weight differs by a factor of about 5 and the rate of total oxidative metabolism per unit weight differs by a factor of about 15. Thus, the ingestion of a given quantity of alcohol per unit body weight does not have the same metabolic significance in man and mouse. To compare the pharmacological impacts, a measurement of intake is not sufficient; one needs to know the resultant concentrations of alcohol in body water over periods of many days.

Monitoring the changes in concentration associated with voluntary ingestion also permits some inferences on motivation. If voluntary consumption of alcohol sustains an intoxicating level in blood, then the drinking behavior would be consistent with a belief that the intoxicating effects of alcohol were reinforcing. In the absence of a functionally significant degree of intoxication it would seem unlikely that the animals had been drinking to achieve this neurological effect.

The present report describes a method to monitor the concentration of alcohol in blood of undisturbed mice drinking voluntarily. The data provide partial answers to the above questions.

MATERIALS AND METHODS

Male mice of the C57BL/6J strain (obtained from The Jackson Laboratory) were chosen because of their genetically determined preference for solutions of alcohol in beverage concentration as an alternative to plain water. Some 30 shipments of these animals have been received in the course of the present experiments, which have extended over a period of 3 years. In general, the animals have shown a pronounced preference for alcohol; the average ratio of 10% alcohol solution to total fluid consumption in different batches has varied from about 60% to 90%.

The mice were housed individually in plastic cages located in a quiet, temperature-controlled (24 ± 1°C) room with a 12 hr:12 hr light/dark cycle. Pelleted Mouse Chow (Purina Lab Chow, no. 5001) was available ad lib. After arrival in the laboratory, weighing 20–25 g (age, about 8 wk), each batch was acclimated to the environment by 2–6 weeks' residence before being used in any experiment.

Details of the equipment developed in our laboratory for continuous monitoring of fluid consumption by mice have been described (18). In brief, each lick on the metal spout of an inverted fluid bottle is detected electronically and registered in a microcomputer. Depending on the design of an experiment, one to three bottles containing different solutions are provided to each mouse, and the lick count at each channel is accumulated over intervals of 1–60 min. The data produced by 144 channels over periods of weeks to months are stored on computer diskettes for subsequent analysis, including conversion of lick counts into volume equivalents by calibration factors. The 144 channels in this system accommodate experiments involving large enough blocks of replicate animals for statistical analyses.

A micromethod for repeated determination of alcohol in

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

blood of mice was needed. The technique of bleeding from the cut tail was found to be seriously inaccurate for study of the rapid changes in concentration of alcohol in blood associated with spontaneous drinking (19). Samples taken from the retroorbital venous plexus (20) into heparinized capillary tubes have proven satisfactory. This procedure is surprisingly well tolerated by the animal and provides reliable samples of blood circulating in metabolically active organs. After the capillary tubes, sealed with wax, are centrifuged to separate plasma, a 0.3-μl sample of plasma is taken into a calibrated microsyringe for injection through a precolumn into a gas chromatograph equipped with a recording integrater (19). Triplicate determinations of plasma alcohol concentration by this method have shown a coefficient of variation of about 3%. Because the volumes of blood are small (5–20 μl), multiple samples can be taken over a period of 1–6 hr without significant change in hematocrit.

To provide a continuous measure of tissue exposure to alcohol during voluntary consumption without disturbing the animals, an indirect method for estimating alcohol in circulating blood has been developed. Equations reflecting the dynamics of intake of alcohol and elimination from the bloodstream were formulated. When the lick/volume ratio, and the rates of absorption, distribution, and elimination have been calibrated by appropriate measurements, the concentration of alcohol in body water can be estimated from the observed rate of consumption of alcohol. In critical experiments, blood samples must be taken periodically for determination of alcohol content and used for adjustment of parameters to individual animals.

A three-compartment model, representing gastrointestinal tract, rapidly mixing body water, and slowly mixing body water, is sufficient for calculation. Suppose that a mouse ingests alcohol at a constant rate (I) during a short time interval (H). If the flux from gut to blood is proportional (K_1) to the quantity of alcohol in gut, then the mean flux, F_m, into the bloodstream during the interval is

$$F_m = I + \{(F_0 - I)\cdot[1 - \exp(-HK_1)]\}/HK_1,$$

in which F_0 is the flux at the beginning of the interval and K_1 is the fractional rate of absorption. Alcohol in blood is equilibrated into total body water at a rate proportional, K_2, to the difference ($C_1 - C_2$) between its concentration in blood and its concentration in the slowly mixing compartments and [as shown by Wilkinson (21)] is eliminated from the body at a rate formally equivalent to Michaelis–Menten kinetics:

$$V_1 \frac{dc_1}{dt} = F_m - K_2(C_1 - C_2) - MC_1/(B + C_1)$$

$$V_2 \frac{dc_2}{dt} = K_2(C_1 - C_2),$$

in which M is maxmial metabolic rate and B is a constant with dimension of concentration (analogous to V_{max} and K_m, respectively, in enzyme kinetics).

These equations of course require sufficiently accurate and stable values of the parameters to be of any use. Fortunately, absorption from the gut and mixing of alcohol into most of body water of the mouse are quite rapid (total half-time, <5 min) relative to the rates of ingestion and metabolism. The second equation therefore can be neglected on a time scale of 10 min or longer. This reduces the problem to a single equation in which all variables are directly measurable:

$$V \frac{dc_1}{dt} = F_m - MC_1/(B + C_1),$$

in which V is total body water ($V = V_1 + V_2$). Deviations from this approximation during the first 10 min after ingestion can be used to estimate parameters in the second equation. Computer programs, based on the Runge–Kutta method of numerical integration (22), have been used for solving the differential equations, and nonlinear least square calculations have given best values for the parameters.

The rapidity of mixing of alcohol into almost all of body water after absorption is a consequence of the rapid circulation of blood in mice. The cardiac output of a resting 30-g mouse is ≈10 ml/min. (The rate of oxygen consumption by the animal is about 1.5 ml/min and the arteriovenous difference in concentration of oxygen cannot be much greater than about 0.15 ml/ml.) Since the total body water in a 30-g mouse is about 20 ml, the circulation of water, and therefore the potential exchange frequency for solutes between blood and body water, is about 50%/min. Likewise, potential exchange frequencies for different tissues can be estimated from the local rates of oxygen consumption. Assuming that the diffusion coefficient of alcohol in tissue water is comparable to that of oxygen, the average half-time of equilibration of alcohol in arterial blood with alcohol in total body water is ≈1.4 min. For the most actively perfused tissues (brain, liver and other visceral organs, and endocrine tissues) the half-time is <1 min. Tail, with limited circulation relative to its water content, is at the other extreme; at usual room temperature the half-time for equilibration of tail water with alcohol in arterial blood may be as long as 30 min (20). Muscle is intermediate in equilibration rate, depending on physical activity. In a mobile unanesthetized mouse, the half-time appears to be no greater than a few minutes.

RESULTS

As illustrated by Fig. 1, the calculated concentration of alcohol in circulating blood fluctuates rapidly with voluntary drinking—a finding confirmed in the present study by several hundred analyses of samples taken at critical times. A wide fluctuation is easily demonstrated in the alcohol-preferring C57BL mice, with a mean daily intake of about 5 mg of alcohol per kg of body weight and a night/day intake ratio of about 4. During almost every night the concentration of alcohol in their blood rises to pharmacologically significant levels, although not for extended periods of time.

Of immediate interest is the association between level of alcohol in blood (and therefore in brain and other active tissues) and subsequent drinking behavior. Surprisingly, there appears to be none. If the pharmacological action of alcohol had been reinforcing, the temporal pattern of drinking should have been better adapted to maintenance of an intoxicating

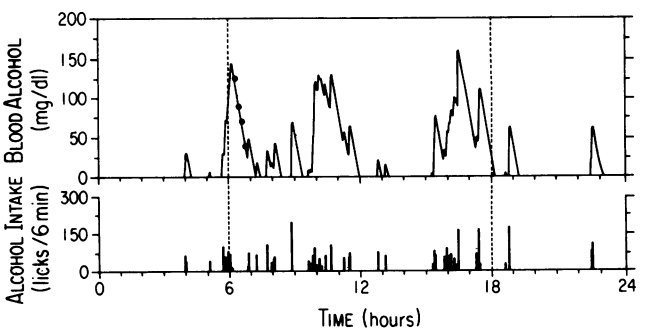

FIG. 1. Typical 24-hr patterns of voluntary consumption and concentration of alcohol in blood plasma of a mouse. The lower panel shows the intake, measured in licks, of 10% alcohol per 6-min period. The continuous curve in the upper panel is the estimated concentration; the four points on this curve (●, between hr 6 and 7) were measured values. The room was dark between 6 and 18 hr.

concentration. On the other hand, if intoxication had been aversive, then the peaks in concentration should have been eliminated by slowing the ingestion of alcohol or by avoiding the substance entirely. In fact, the animals did neither. Their patterns of drinking appear to reflect a taste preference for alcohol in beverage concentration, without regard to its pharmacological effects. This conclusion is supported by previous experiments in which clearance of alcohol was retarded by administration of the alcohol dehydrogenase inhibitor 4-methylpyrazole. Animals so treated continued to drink considerable amounts of alcoholic solution, despite exceptionally high and sustained concentrations of alcohol in blood (23).

Further evidence against a pharmacological motive in consumption of alcohol is given by the statistical association between intakes of alcoholic solutions and plain water. Although the total fluid intake (sum of alcoholic solution and water) varies from hour to hour, the intakes of the two fluids generally vary in parallel. If alcohol were being consumed for its pharmacological effect, one would expect its intake to be dissociated in time from the homeostatically determined need for water. This was not the case in the animals that we have observed.

The cumulative exposure of internal tissues to alcohol can be defined by a measure that reflects both intensity and duration. Using the calculated concentrations of alcohol in plasma as a function of time, one can compute the cumulative statistical distribution in time of exposure to various concentrations. Fig. 2 shows the estimated number of hours within the 24-hr cycle during which alcohol was at or above the concentration shown on the abscissa. From the perspective of an experimenter, this curve also shows the probability of finding a concentration of alcohol at or above any given level if a sample of blood is taken at a random time. Since the biological effects of toxins differ for different temporal patterns of exposure, it seems likely that a knowledge of this statistical distribution will be needed for analysis of events at the molecular level. As a practical matter, it is evident from the rapid fluctuations in concentration that occasional samples of blood taken from mice are an inadequate measure of the toxic effects of voluntary drinking.

Fig. 2 shows the markedly different degrees of cumulative exposure in mice and men. The curves in the lower left quadrant of the diagram were calculated from data on six typical mice voluntarily ingesting alcohol at a daily rate of 4–6 g/kg. The curves in the upper region were derived from data on four severely alcoholic men who were permitted to drink 80 proof Vodka, ad lib, up to but not exceeding 1 liter per day for a limited time on a metabolic ward (personal communication, Enoch Gordis). Under these conditions they consumed 3.5–4.3 g/kg per day. Because of the lower rate of clearance by man relative to the C57 mouse [23 mg/dl per hr for man (24) vs. 109 mg/dl per hr for mouse (unpublished data)], the tissues of alcoholics were exposed to alcohol at much higher concentration than the tissues of mice, despite the lower dose taken by man. Mice, therefore, are protected from acute toxicity by high rate of clearance, even when consuming very large doses of alcohol.

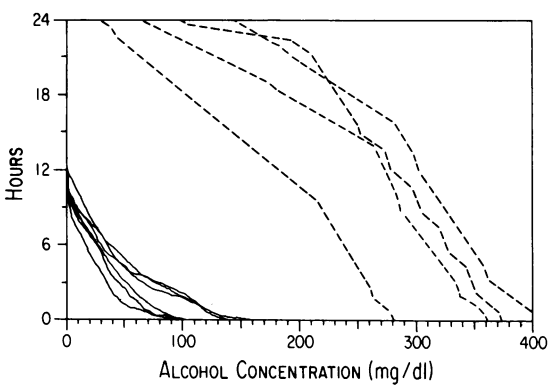

FIG. 2. Exposure of internal tissues to alcohol during a 24-hr period of voluntary consumption. The curves are cumulative statistical distributions of plasma alcohol concentration over time. They show the number of hours that alcohol in plasma was at or above the concentration indicated in the abscissa. The curves in the lower left quadrant were obtained from data on six typical mice with a pronounced preference for alcohol. The curves in the upper region were calculated from data on four alcoholic men who were permitted to drink, ad lib, on a metabolic ward for 18 of the 24 hr.

Man is further disadvantaged relative to mouse in chronic exposure. Although the rate of clearance of alcohol from blood is less for man than for mouse when calculated per unit volume of plasma (or per unit body weight), it is significantly higher in man when oxidation of alcohol is related to total oxidative metabolism. The alcoholic man thus is in double jeopardy. Not only is he at hazard from the acute toxic effects of alcohol because of slow clearance from body water but he is also in danger of metabolic overload after clearing the substance since the metabolites of alcohol must pass through the oxidative chain before final elimination. The ratio of maximal clearance of alcohol (expressed in calories) to basal metabolic rate (in the same units) is about 0.6 for man and 0.2 for mouse.

These discrepancies in scale factors obviously preclude a simple modeling of alcoholic toxicity in mice. Although the toxicity of sustained concentrations of alcohol can be demonstrated in mice if intake is forced and clearance is inhibited, the reduction in clearance rate reduces the probability of overload by metabolites, which in the long term may be the more serious clinical hazard. Indeed, the accelerated metabolism of alcohol by alcoholics, while diminishing acute effects, may in fact enhance the danger of liver disease; when an alcoholic acquires an increased capacity to clear alcohol, he also becomes able to produce a greater excess of acetate to distort metabolism and promote accumulation of fat in liver. Mice are unsuitable subjects for modeling this phenomenon, which can be observed in primates (25, 26) with a ratio of alcohol clearance to metabolic rate more comparable to that of man.

DISCUSSION

Because mice can consume so much greater quantities of alcohol without toxic effects, it becomes necessary to ask whether this consumption is relevant to the concerns of clinicians treating alcoholics. Ingestion of a substance as a food or flavor is of little interest medically if it does no harm. Preference without toxicity does not constitute a model of alcoholism (27).

Nevertheless, the central question in the disease of alcoholism is the motivation for drinking. This can be studied to some extent in mice, and the findings might be relevant to human behavior. Is alcohol consumed as a food or a drug? More precisely, is the pharmacological effect of alcohol reinforcing under any circumstances? If conditions can be found in which some mice drink large amounts of alcohol at abnormal times of day and in such a pattern as to sustain an intoxicating blood level, it might be inferred that the motivation for drinking was pharmacological rather than homeostatic. The mice that we have studied so far have not shown this behavior. They have consumed alcohol during the normal nocturnal episodes of eating and drinking. Although the concentration of alcohol in their blood frequently reached intoxicating levels, the exposure was not sustained. During the 12-hr period of light in the room, consumption of alcohol was minimal and only trace concentrations could be found in circulation.

The failure of the mice to sustain a pharmacologically effective concentration of alcohol in blood cannot be attributed to an inability to drink a sufficient volume of 10% alcohol. Similar mice have been observed to consume much greater volumes of 5% sucrose or 0.1% saccharine. In fact, if the mice in the present study had ingested as much 10% alcohol as those ingesting these more highly preferred solutions, their intake of alcohol would have exceeded capacity for clearance and the concentration in blood would have risen in a few hours to the level of coma and death.

Although the discrepancy in metabolic scale factors appears to rule out the possibility of defining alcoholism in mice by the same criteria as in man, an alternative definition of abnormal drinking is possible and this encourages us to continue the search for conditions under which it might occur. With continuous monitoring of the consumption of alcohol when animals have unlimited access to plain water and alcohol in beverage concentrations, with no restriction on food, and with continuous estimation of the concentration of alcohol in body water, nonhomeostatic drinking of alcohol should become apparent. Consumption of substantial quantities at abnormal times, uncorrelated with the normal pattern of eating and drinking, would suggest a pharmacological determinant. If the atypical pattern of intake is specific for alcohol and sustains an intoxicating concentration in blood, the inference of alcoholic-type drinking would be strengthened. With these criteria it is possible that models can be found with greater relevance to the compulsive drinking of humans than those obtained by selecting animals that simply like the taste of the beverage.

Professor Enoch Gordis (Mt. Sinai Medical School) kindly provided data from his metabolic studies of alcoholic men. The work was supported in part by a grant from the John L. and Helen Kellogg Foundation.

1. Lester, D. (1961) *Q. J. Stud. Alcohol* **22,** 223–231.
2. Freund, G. (1969) *Arch. Neurol.* **21,** 315–320.
3. Lieber, C. S. & DeCarli, L. M. (1970) *J. Biol. Chem.* **245,** 2505–2512.
4. Goldstein, D. B. & Pal, N. (1971) *Science* **172,** 288–290.
5. Mello, N. K. & Mendelson, J. H. (1971) *Physiol. Behav.* **7,** 827–836.
6. Eriksson, K. (1972) *Finn. Found. Alcohol Stud.* **20,** 121–125.
7. Falk, J. L., Samson, H. H. & Winger, G. (1972) *Science* **177,** 811–813.
8. Hunt, W. A. (1973) *Neuropharmacology* **12,** 1097–1102.
9. Roach, M. K., Khan, M. M., Coffman, R., Pennington, W. & Davis, D. L. (1973) *Brain Res.* **63,** 323–329.
10. Pohorecky, L. A. (1974) *J. Pharmacol. Exp. Ther.* **189,** 380–391.
11. Meisch, R. A. & Thompson, T. (1974) *Psychopharmacology* **37,** 311–321.
12. Freed, E. X. (1974) *Q. J. Stud. Alcohol* **35,** 1035–1043.
13. Myers, R. D. & Melchior, C. L. (1977) *Science* **196,** 554–556.
14. Belknap, J. K., Berg, J. H. & Coleman, R. R. (1978) *Pharmacol. Biochem. Behav.* **9,** 1–6.
15. Li, T.-K., Lumeng, L., McBride, W. J., Waller, M. B. & Hawkins, T. D. (1979) *Drug Alcohol Depend.* **4,** 45–60.
16. Lumeng, L., Waller, M. B., McBride, W. J. & Li, T.-K. (1982) *Pharmacol. Biochem. Behav.* **16,** 125–130.
17. Richter, C. P. (1941) *Q. J. Stud. Alcohol.* **1,** 650–662.
18. Dole, V. P., Ho, A. & Gentry, R. T. (1983) *Physiol. Behav.* **30,** 971–974.
19. Riley, V. (1960) *Proc. Soc. Exp. Biol. Med.* **104,** 751–754.
20. Gentry, R. T., Rappaport, M. S. & Dole, V. P. (1983) *Physiol. Behav.* **31,** 529–532.
21. Wilkinson, P. K. (1980) *Alcohol. Clin. Exp. Res.* **4,** 6–21.
22. Romanelli, M. J. (1960) in *Mathematical Methods for Digital Computers*, eds. Ralston, A. & Wilf, H. S. (Wiley, New York), pp. 110–120.
23. Gentry, R. T., Rappaport, M. S. & Dole, V. P. (1983) *Alcohol. Clin. Exp. Res.* **7,** 420–423.
24. Wilkinson, P. K., Sedman, A. J., Sakmar, E., Earhart, R. H., Weidler, D. J. & Wagner, J. G. (1976) *Clin. Pharmacol. Ther.* **19,** 213–223.
25. Rubin, E. & Lieber, C. S. (1974) *N. Engl. J. Med.* **290,** 128–135.
26. Lieber, C. S., DeCarli, L. M. & Rubin, E. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 437–441.
27. Cicero, T. J. (1980) in *Animal Models in Alcohol Research*, eds. Erikkson, K., Sinclair, J. D. & Kiianmaa, K. (Academic, New York), pp. 99–117.