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Temperature Affects Ethanol Lethality in C57BL/6, 129, LS and SS Mice

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FINN, D. A., M. BEJANIAN, B. L. JONES, P. J. SYAPIN AND R. L. ALKANA. *Temperature affects ethanol lethality in C57BL/6, 129, LS and SS mice.* PHARMACOL BIOCHEM BEHAV **34**(2) 375–380, 1989.—The effect of ambient and body temperature on ethanol lethality in inbred strains and selected lines of mice was investigated. C57BL/6J, 129/J, LS/Ibg and SS/Ibg mice were exposed to 23 or 34°C following IP injection of lethal ethanol doses (8.2, 6.0, 6.5 or 7.0 g/kg ethanol, respectively). All mice exposed to 23°C during intoxication became markedly hypothermic, with mean body temperatures dropping to lows of 27.9, 30.0, 33.0 and 33.3°C in C57, LS, SS and 129 animals, respectively. Compared to the 23°C groups, exposure to 34°C offset the ethanol-induced hypothermia and significantly increased percent mortality in all four mouse genotypes. Exposure to 34°C increased mortality at 24 hours postinjection from 15% to 95% in SS mice, from 37.5% to 100% in 129 mice and from 50% to 100% in LS and C57 mice. Blood ethanol data suggest that the present results cannot be explained by temperature-related changes in ethanol elimination. These results provide further evidence that body temperature during intoxication can have major effects on mortality rates in mice.

Body temperature Ethanol lethality Inbred strains of mice Selected lines of mice

MANIPULATION of body temperature during intoxication can strongly influence the sensitivity of animals to ethanol's behavioral and toxic effects (1–3, 8, 10, 12, 14, 19, 23, 25–27, 33). Studies in mice indicate that the potency of lethal ethanol doses decreases as body temperature is decreased by ambient temperature challenge during intoxication (8, 14, 23). The most extensive study found that the 8-hour (hr) LD_{50} in C57 mice increased from 5.3 g/kg in the 35°C exposed animals to 8.7 g/kg in the 20°C exposed mice (23). The 35°C environment offset ethanol-induced hypothermia, while body temperatures in the 20°C exposed mice continued to drop to a mean of 22.6°C at 8 hr postinjection. These results agree with and extend earlier studies that indirectly suggested a link between temperature and ethanol lethality (8, 14, 16, 20). In addition, drugs which produce hypothermia pharmacologically have been shown to protect against acute ethanol toxicity (12), providing further support for the hypothesis that a decrease in body temperature has a protective effect against ethanol lethality.

Recent work suggests that genetic background influences the qualitative effect of body temperature manipulation on ethanol sensitivity following hypnotic doses of ethanol (1,2). In general, offsetting hypothermia increased sensitivity to ethanol-induced loss of righting reflex (LORR) duration in C57 and short-sleep (SS) mice and decreased sensitivity to ethanol-induced LORR duration in the 129 and long-sleep (LS) mice. The present study was conducted to determine whether offsetting hypothermia would also differentially affect sensitivity to lethal ethanol doses in the 129, C57, LS and SS mice.

METHOD

Drug-naive LS/Ibg and SS/Ibg male mice were obtained from the Institute for Behavioral Genetics in Boulder, CO. The C57BL/6J and 129/J male mice were purchased from Jackson Laboratories. Upon arrival, the mice were separated by genotype, housed 4/cage and acclimated to a 12-hr light/dark cycle (0700 on) in a room thermostatically maintained at 23 ± 1°C for a minimum of 1 week before experimentation. Mouse food pellets (Wayne Lab Blox) and water were freely available.

Between 1000 and 1030 hr, baseline rectal temperatures were measured using a digital thermometer (Bailey Instruments Co., Saddle Brook, NJ, Model BAT-12) and glycerol lubricated temperature probe (Bailey Instruments Co., Model RET-3) that was inserted 1.9 cm into the rectum. Immediately following baseline temperature measurements, the mice were injected intraperitoneally (IP) with ethanol (20% w/v solution in normal saline). The dose for each genotype was chosen on the basis of pilot studies to cause approximately 50% mortality at 24 hr postinjection in animals exposed to normal room temperature (22–23°C). The doses of ethanol were 8.2 g/kg for C57 mice, 6.0 g/kg for 129

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mice, 6.5 g/kg for LS mice and 7.0 g/kg for SS mice. The doses given to the LS and SS mice are higher than in previous studies involving earlier generations (9), but are consistent with recent work indicating that the LD_{50}s in these mice are changing with continued selection pressure (4).

Immediately following injection, the mice were placed into 9×6 cm compartments within a standard cage. The entire cage, containing 5 singly housed mice, was then placed into a temperature-controlled chamber (Model CEC 50 LPT, Puffer Hubbard, Weaverville, NC) and exposed to ambient temperatures of 23°C or 34°C for 24 hr. A single ambient temperature was tested each day in separate groups of animals. Both the LS and SS mice were tested simultaneously during each test day to control for possible day to day variability. The C57 and 129 mice were tested together in a separate experiment. Mortality was determined at 1, 2, 4, 8 and 24 hr postinjection. Death was defined as the absence of respiration for one minute. Rectal temperatures were measured in the surviving animals immediately following the mortality determination.

In a separate study mice from each genotype were treated as described above. A 20 μl blood sample was taken from the orbital sinus (29) in surviving animals at 30, 60, 120 and 180 minutes postinjection. The blood samples were prepared and frozen for subsequent analysis of ethanol concentration using headspace gas chromatography (24).

Data Analysis

The present study utilized percent (%) mortality as a means of comparing the effect of body temperature manipulation on lethality from ethanol. This method has been previously used in our laboratory to demonstrate body temperature effects on ethanol lethality in inbred strains. The use of inbred strains allowed a clear assessment of the effects of temperature in the absence of genetic variation. Further, the % mortality approach greatly reduces the number of animals required compared to a complete LD_{50} analysis.

Construction of survival curves and life tables was used to compare mortality rates during the 24-hr period across ambient temperatures. The Mantel-Haenszel Method of Survival Analysis, which controls for time effects, was used to determine the overall effect of ambient temperature on survival (22). Application of the Mantel-Haenszel method results in a chi-square value with one degree of freedom. This chi-square statistic permits simultaneous comparison across all time intervals of the differences in survival probabilities for the two ambient temperature groups.

An ethanol elimination rate for each group was calculated by conducting one linear regression on all the blood ethanol data for that group. Elimination rates for individual mice could not be calculated due to the large number of deaths, particularly in the animals exposed to 34°C. The slopes obtained from linear regression for each ambient temperature group within each strain were compared using a *t*-test for comparisons among slopes (35).

The rectal temperature data for the 23°C and 34°C groups within each genotype was compared to the respective baseline temperature data by two-tailed pairwise *t*-tests. Analysis of variance could not be conducted due to the number of empty cells in the 34°C groups.

A p value of <0.05 was taken as significant.

RESULTS

Survival analysis indicated that there was a statistically significant effect of ambient temperature on survival in the LS ($\chi^2 = 25.504$, $p<0.001$), SS ($\chi^2 = 21.863$, $p<0.001$), 129 ($\chi^2 = 4.629$, $0.01<p<0.05$) and C57 ($\chi^2 = 22.621$, $p<0.001$) mice (Fig. 1). In the LS and C57 mice exposed to the 23°C environment, 50% of the animals survived 24 hr postinjection. In contrast, there were no C57 or LS animals surviving by 2 and 4 hr postinjection, respectively, in the 34°C environment. Survival in the 129 mice decreased from 62.5% in the 23°C environment at 24 hr postinjection to 0% by 8 hr postinjection in the 34°C environment. At 24 hr postinjection in the SS animals, the percentage of animals surviving decreased from 85% in the 23°C exposed animals to 5% in the 34°C exposed mice. Therefore, not only did the 34°C environment dramatically increase lethality in all animals, but the lethality also occurred at an earlier time in three of the four genotypes of mice.

All mice exposed to the 23°C environment after ethanol injection became markedly hypothermic (Fig. 2). Rectal temperatures remained fairly constant across time in LS, SS and 129 mice, with mean body temperatures dropping to lows of 30.0, 33.0 and 33.3°C, respectively, within the first 2–4 hr after ethanol. The C57 mice had the greatest degree of hypothermia. Mean body temperature in this strain continued to drop and reached a low of 27.9°C at 8 hr postinjection. In contrast, exposure to 34°C offset ethanol-induced hypothermia in all mice, and caused mild hyperthermia in the C57 and 129 inbred strains.

There was also a significant effect of ambient temperature on the ethanol elimination rate (Fig. 3). The LS and SS mice exposed to 34°C during intoxication eliminated ethanol more rapidly than similarly treated animals exposed to 23°C. In the SS mice there was a statistically significant increase in the ethanol elimination rate from 0.87 mg/ml/hr at 23°C to 1.39 mg/ml/hr at 34°C. A similar, but nonsignificant, increase in ethanol elimination rate from 0.69 mg/ml/hr at 23°C to 1.16 mg/ml/hr at 34°C was seen in the LS mice. The elimination rate in C57 mice exposed to 23°C was 0.50 mg/ml/hr. The elimination rate for similarly treated C57s exposed to 34°C was not determinable, since all the animals in this group were dead by 1 hour postinjection. In the 129 mice there was a nonsignificant decrease in ethanol elimination rate from 0.67 mg/ml/hr at 23°C to 0.59 mg/ml/hr at 34°C.

DISCUSSION

Ambient temperature significantly affected rectal temperature and survival in all four genotypes of mice tested. All animals exposed to 23°C during intoxication became markedly hypothermic and had respective survival rates of 50%, 50%, 62.5% and 85% at 24 hr postinjection in the C57, LS, 129 and SS mice. In contrast, exposure to 34°C offset ethanol-induced hypothermia and significantly decreased survival to 0% in the C57, LS and 129 mice and to 5% in the SS mice. These results agree with previous studies conducted in inbred strains of mice (8, 12, 14, 23), which demonstrated an increase in lethal ethanol sensitivity as body temperature during intoxication was manipulated between 32–38°C. The present results also extend previous work to a broader range of genetic backgrounds, including mice selectively bred for differences in sensitivity to the hypnotic effects of ethanol.

The consistent increase in sensitivity to lethal doses of ethanol as hypothermia was offset in the present study contrasts with the differential responses to body temperature manipulation following hypnotic ethanol doses in these genotypes (1,2). Specifically, offsetting hypothermia increased ethanol sensitivity, measured by duration of LORR and ethanol concentrations at the return of the righting reflex, in C57 and SS mice, and decreased ethanol sensitivity in the 129 and LS mice. These results with hypnotic and lethal ethanol doses suggest that the effects of body temperature manipulation on ethanol sensitivity may be dose dependent as well as genetically determined. The findings also suggest that there

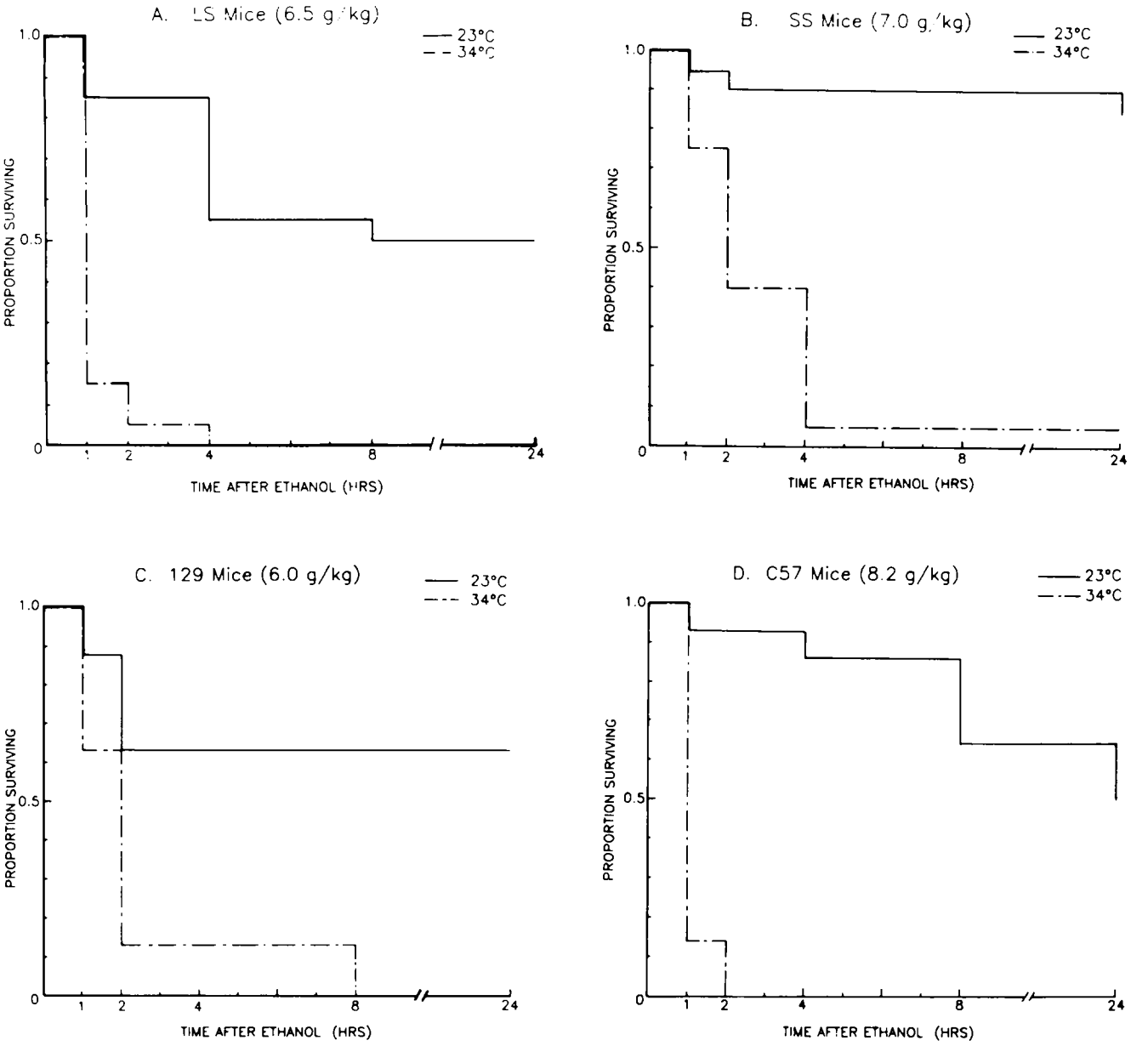

FIG. 1.The effect of ambient temperature on ethanol lethality in (A) LS mice, (B) SS mice, (C) 129 mice and (D) C57 mice. Shown are the proportion of animals surviving during the 24-hr period following injection of ethanol and exposure to 23°C or 34°C. Survival analysis indicated that there was a statistically significant effect of ambient temperature on survival in all four genotypes tested. See the Results section for specific statistical analysis. The number of animals for each ambient temperature group began at n = 20 for the LS and SS mice, n = 14 for the C57 mice and n = 8 for the 129 mice.

may be mechanistic differences between the causes of intoxication and lethality from ethanol.

The similar qualitative effect of offsetting hypothermia following lethal ethanol doses in four genetically distinct mouse inbred strains and selected lines suggests that a common mechanism may mediate the effect of body temperature manipulation on ethanol lethality. Therefore, the decrease in ethanol lethality which accompanied hypothermia could involve an interaction of temperature with the membrane actions of ethanol (11, 13, 32). In vitro data (6,15) has shown that decreasing membrane temperature can decrease the ethanol-induced perturbation of brain membranes which are held to underlie the depressant effect of ethanol on brain function. However, whether decreasing temperature affects ethanol-induced lethality by altering ethanol's initial action in the membrane, neurotransmitter or receptor function (18), or other aspects of physiological function, such as a decrease in oxygen requirement (28), remains to be determined.

Temperature-related changes in the pharmacokinetics of ethanol have been shown to occur, but they do not appear to mediate the effects of temperature on ethanol lethality. In the present study, exposure to 34°C significantly increased the ethanol elimination rate in SS mice, with a nonsignificant increase in elimination rate in LS mice and a nonsignificant decrease in 129 mice. The lack of significance in LS mice may reflect the large number of deaths at later time points in the 34°C group, and the resultant limitation in the regression analysis. Nonetheless, an increase in

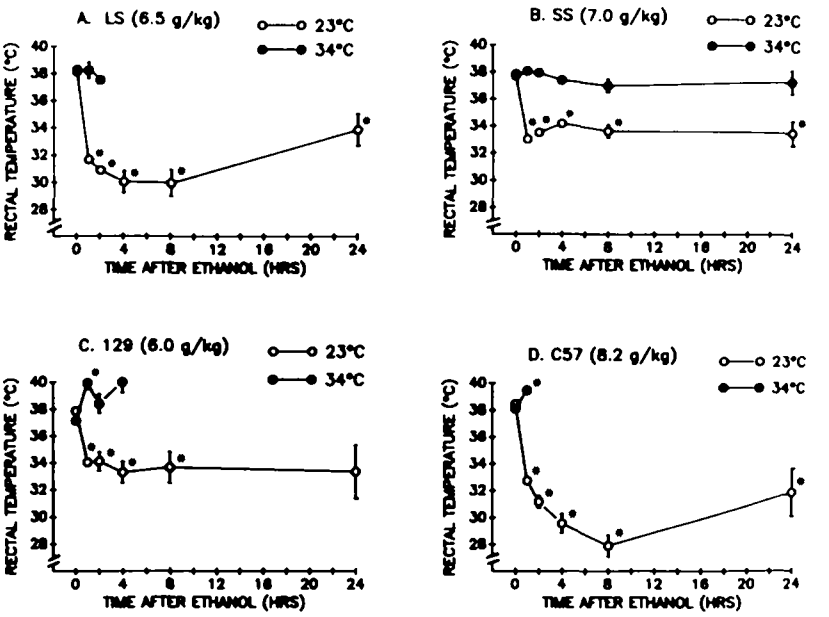

FIG. 2. The effect of ambient temperature on intoxicated rectal temperature in (A) LS mice, (B) SS mice, (C) 129 mice and (D) C57 mice. Shown are the mean ± SE rectal temperatures for the surviving animals depicted in Fig. 1. All mouse genotypes exposed to the 23°C environment became markedly hypothermic, whereas the 34°C environment offset the ethanol-induced hypothermia. In cases where the SE is not visible, it is contained within the symbol. $*p<0.05$, two-tailed pairwise *t*-test vs. respective baseline rectal temperature.

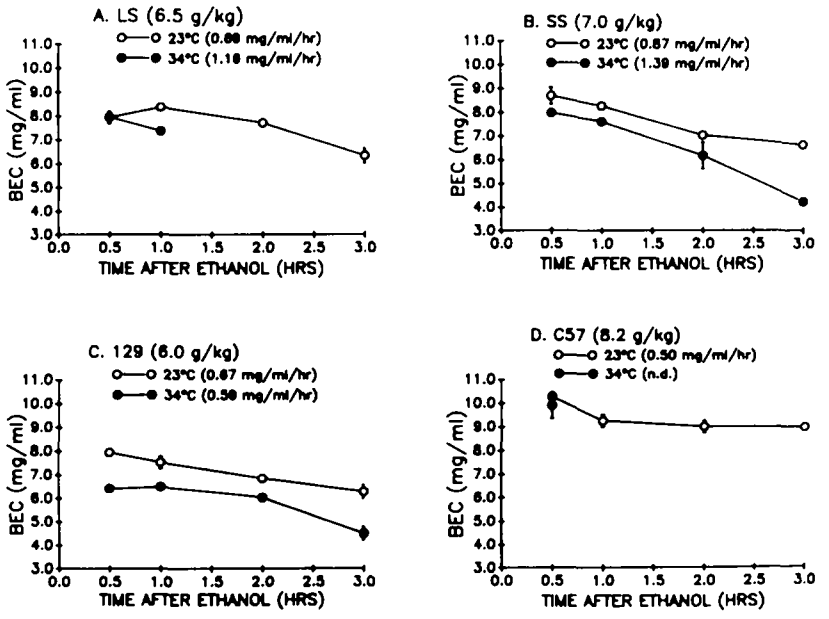

FIG. 3. The effect of ambient temperature on blood ethanol concentration (BEC) and ethanol elimination rate in (A) LS mice, (B) SS mice, (C) 129 mice and (D) C57 mice. Shown are the mean ± SE blood ethanol concentration (BEC) in separate groups of mice treated similarly to those depicted in Fig. 1. In cases where the SE is not visible, it is contained within the symbol. The number of animals for each ambient temperature group began at n = 10 for LS and SS mice, n = 8 for C57s and n = 5 for 129s. The ethanol elimination rate for each ambient temperature group within each genotype is shown in parentheses. See the Results section for statistical comparisons. (n.d. = not determinable due to ethanol lethality.)

ethanol elimination rate seen in both the LS and SS mice at warm vs. cooler body temperatures is consistent with previous work in these animals which demonstrated that as the dose of ethanol increased, the body temperature and ethanol elimination rate decreased by approximately 50% (31). The elimination rate for C57s exposed to 34°C in the present study was not determinable. However, previous work has shown that an increase in body temperature during intoxication in C57 mice is associated with an increased ethanol elimination rate of 50–60% (5,31). A temperature-dependent increase in ethanol elimination in the 34°C exposed C57, LS and SS mice should cause a decrease, rather than an increase in ethanol lethality. In addition, the mean blood ethanol concentration at all times measured was equivalent or lower in the 34°C groups when compared to the 23°C groups (Fig. 3), further indicating that altered pharmacokinetics were not responsible for the increase in mortality at 34°C.

Interestingly, previous work has shown that an aldehyde dehydrogenase inhibitor, cyanamide, increased acetaldehyde levels and decreased the LD_{50} of ethanol (17), suggesting that acetaldehyde accumulation can potentiate ethanol toxicity. Although acetaldehyde concentrations were not determined in the present study, it is unlikely that a 50% increase in the ethanol elimination rate could result in the high acetaldehyde levels which were associated with increased mortality in the cyanamide study (17).

Hyperthermia per se in the mice exposed to 34°C during intoxication did not appear to contribute to the higher mortality in these animals. Earlier studies have reported lethal temperatures (LT_{50}s) for mice to be 41.8°C (8), 42.0°C (34) and 43.3°C (28). In the present study the LS and SS mice exposed to 34°C were not hyperthermic, while the C57 and 129 mice exposed to 34°C became slightly hyperthermic. Mean body temperatures in the 129 and C57 animals at 1 hr postinjection were 39.9°C and 39.5°C, respectively. Rectal temperature in the one surviving 129 mouse at 4 hr postinjection was 40.0°C. These rectal temperatures are lower than the reported LT_{50} rectal temperatures for the mouse (8, 28, 34), and suggest that the increased lethality in the 34°C exposed animals was due to an increased sensitivity to ethanol, rather than an effect of hyperthermia per se.

Overall, the present findings combine with previous work (8, 12, 14, 16, 20, 23) to demonstrate that body temperature markedly influences sensitivity to ethanol lethality across a spectrum of genetic backgrounds. The consistent effect of temperature on ethanol lethality in C57, 129, LS and SS mice contrasts with the genetically determined differences in the qualitative interaction between temperature and sensitivity to the hypnotic effects of ethanol in these mice (1,2). Clinically, these results support the hypothesis (23) that holding body temperature constant at a subnormal level may represent a simple, noninvasive means of enhancing existing supportive measures (7, 21, 30) and further reducing mortality from ethanol overdose.

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