processes. However, our research establishes that *H. moorei* is a remarkable exception, since this sponge accumulates and maintains a quantity of potassium fluorosilicate equal to about 5 percent of its wet (living) weight. The slight solubility of potassium fluorosilicate in water (0.77 g/liter at 0°C; 1.77 g/liter at 25°C) and in some other hydrous systems (*11*) indicates that most of it must be present in *H. moorei* in the solid state. Whether it constitutes an essential part of the skeletal tissue along with the amorphous silica also shown to be present by x-ray diffraction has yet to be determined quantitatively. Conventional procedures for separating spicules from sponge tissue involve solubilizing the tissue with aqueous oxidants and are, of course, unsuitable for the quantitative analysis of a water-soluble compound such as potassium fluorosilicate. In our qualitative examination of *H. moorei*'s spicules, we found nothing to distinguish them from the common siliceous spicules. Electron microscopy studies showed them to be very similar to *H. perleve*'s spicules and typically siliceous. In addition, several treatments of a sample of *H. moorei* with sodium hypochlorite solution produced a residue rich in spicules that appeared to be identical to those in the intact organism.

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Conditioned Tolerance to the Hypothermic Effect of Ethyl Alcohol

Abstract. *Results from experiments with rats support the proposition that tolerance to the hypothermic effect of alcohol involves the Pavlovian conditioning of compensatory responses. Tolerance was substantially reduced when alcohol was administered in an environment that had not been associated with alcohol. Direct evidence of a conditioned hyperthermic compensatory response was found.*

In a series of investigations, Siegel (*1*) proposed an unconventional theory of tolerance (the reduced responsivity to a drug caused by repeated experience with it) to several effects of morphine. Siegel postulated that tolerance to morphine is the algebraic summation of its unconditioned pharmacological effects and a compensatory conditioned response to those effects. According to the theory, the manifestation of tolerance is tied to environmental cues that become associated with the administration of the drug. There is now considerable evidence in support of this theory as it applies to the analgesic and hyperthermic effects of small (5 mg/kg) doses of morphine (*1*). An important test of the generality of Siegel's theory involves its applicability to nonopiate drugs. In this report we present data to show that tolerance to the hypothermic effect of alcohol also involves Pavlovian conditioning, and we give direct evidence for the existence of a conditioned compensatory response.

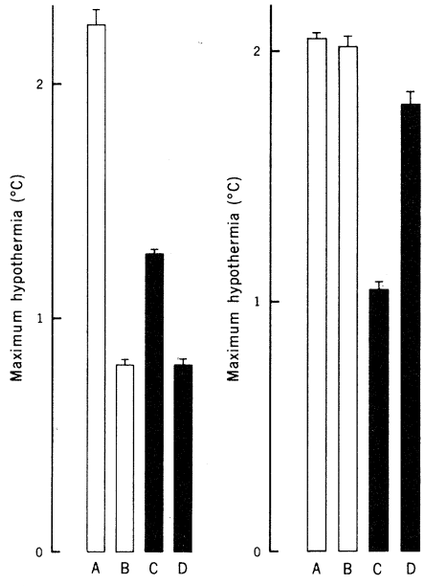

Fig. 1 (left). Peak hypothermic response (mean ± standard deviation) to alcohol in the first experiment. (Bar A) Initial response to alcohol (2.5 mg/kg). (Bar B) Diminished response after nine exposures to alcohol in the distinctive environment. (Bar C) Loss of tolerance during the first exposure to alcohol in the home room. (Bar D) Reinstatement of tolerance in the distinctive environment. Fig. 2 (right). Peak hypothermic response to alcohol (mean ± standard deviation) in the second experiment. (Bars A and B) Response to alcohol in the home (A) or distinctive (B) room when previous experience was with saline. (Bar C) Response of rats given nine injections of alcohol in the distinctive environment and tested for tolerance in the distinctive environment. (Bar D) Response of rats given nine injections of alcohol in the distinctive environment and tested for tolerance in the home environment.

In a preliminary experiment, in which within-subject comparisons were made, nine male Wistar rats (300 to 320 g at the beginning of the experiment) were used. They were caged separately in an environment that was maintained at 21° to 23°C with a photoperiodic cycle of 12 hours of light and 12 hours of darkness. Continuous access to water and laboratory chow was permitted. During tolerance acquisition (18 days), all animals received an intraperitoneal injection of alcohol (2.5 g/kg, 12.5 percent, weight to volume) in isotonic saline on alternate days. On such days, the rats were transported in a cage rack to a room made distinctive by dim illumination and the sound of static from a radio. They were weighed, had their rectal temperature taken as a baseline, and were injected with alcohol. Rectal temperature was again measured at 45, 60, and 75 minutes after the injection. On the days in which alcohol was not administered, the animals remained in their home room, where each was weighed, given an injection of isotonic saline as a control measure, and returned to its cage; no temperatures were taken.

On day 20 of the experiment, all the rats were subjected to the alcohol treatment and temperature measurement procedure, except that for the first time this was done in the home room, where the rats had never experienced the effects of alcohol. On day 22, all the animals were injected with alcohol in the distinctive room. (Days 19 and 21 were typical nonalcohol days.)

The results are displayed in Fig. 1, which shows the mean maximum hypothermia (maximum decrease from baseline) attained under the various experimental conditions. Bar A shows the hypothermia recorded during the first exposure of the rats to alcohol (during tolerance acquisition in the distinctive

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environment). Bar B indicates that considerable tolerance had developed in rats given alcohol in the distinctive environment by the ninth injection (Student's $t = 6.09$, $P < .001$). The most critical data are displayed as bars C and D. Bar C shows the results of the first alcohol test on day 20 in the home environment (which until then had not been paired with the effect of alcohol); the loss of tolerance was substantial (for bar B compared to bar C, $t = 5.82$, $P < .001$). When the animals' thermic response was tested on day 22 in the distinctive (tolerance) environment (bar D), tolerance was immediately reinstated (for bar C compared to bar D, $t = 5.87$, $P < .001$).

Thus the conditioning theory of tolerance was confirmed for a nonopiate such as alcohol at a dose large enough to produce substantial motor impairment in rats (*2*). Tolerance was acquired and maintained in an environment that was repeatedly paired with the systemic effects of alcohol, but tolerance diminished in an environment not previously associated with alcohol.

A second experiment, in which between-group comparisons were made, extended these results and included a control for the possibility that some intrinsic property of the environments, rather than some conditioned effect associated with one of them, might have influenced the results of the first experiment. Each rat in two groups of nine received nine injections of alcohol in the distinctive environment according to the schedule and procedures of the first experiment. Two additional groups of six rats each were treated identically, except that saline was substituted for alcohol. On the test day all of the rats received an injection of alcohol. Each rat in one of the alcohol-treated groups received its tenth injection in the distinctive environment (where it had received the other nine). Each rat in the other alcohol-treated group received its tenth injection in the home room for the first time. One of the saline-treated groups received its first injections of alcohol in the distinctive environment; the other saline-treated group received alcohol for the first time in the home room.

The results are shown in Fig. 2. There was no significant difference in results between the saline-treated groups (bars A and B) that received alcohol for the first time on the test day (for bar A compared to bar B, $t = 0.45$); that is, the environmental factor did not exert any nonassociative effect on the rats' thermic reaction to alcohol. In contrast, the alcohol-treated group that received the drug in the home enviroment for the first time (bar D) was clearly less tolerant than its counterpart (bar C; for bar C compared to bar D, $t = 7.91$, $P < .001$). These groups had the same pharmacological history and had acquired identical levels of tolerance by the ninth acquisition day (mean maximum hypothermic response was 1.1°C for each group). The only factor that differentiated the responses was whether the test environment had been previously associated with the effects of alcohol.

All of the rats that had been made tolerant were left undisturbed in the home room for 10 days and then were given four injections of alcohol (administered on alternate days) in the distinctive environment to reestablish a stable degree of tolerance. On the basis of the thermic response to the fourth injection, animals were assigned in equal numbers to groups matched for tolerance (1.04 compared to 0.94; $t = 0.51$; P not significant) in order to test for the presence of a conditioned compensatory response. This was done by exposing the rats to an injection of saline in the distinctive environment, where tolerance had been established, or in the home room, where it had not.

Evidence for a conditioned compensatory response was also obtained. In the saline placebo test, the peak temperature change for animals tested in the distinctive environment was 0.52°C compared to a change of −0.23°C in the home environment. The difference was significant ($t = 2.76$, $P < .02$). Thus compensatory hyperthermia was evident in the environment associated with tolerance development and not evident in the one in which tolerance had not been acquired, despite identical histories of exposure to alcohol in the two groups.

Clearly, the manifestation of tolerance to the hypothermic effect of a substantial dose of alcohol was conditional upon the environmental cues present at the time that the drug's effect was experienced (*3*). These and Siegel's findings indicate that a conditioning model of tolerance has considerable generality. Moreover, we have provided direct support for the hypothesis that hyperthermia is a conditioned compensatory mechanism that mediates tolerance to alcohol. The conditional elicitation of a compensatory response in heavy drinkers and alcoholics (who have an elevated tolerance for alcohol) could provide the somatic basis for the subjective phenomenon of craving, which has been thought to account for the perpetuation of drinking in such persons (*4*).

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