Reduction in Ethanol Self-Selection of C57BL/6j Mice During Treatment with 3-((2-Imidazoline-2yl)Methyl) Indole (36645)

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Drugs which inhibit ethanol self-selection in animals (1) may also reduce intake of other preferred fluids such as saccharin solutions (2). Some drugs produce persistent rejection of ethanol after the drug is withdrawn (3, 4), suggesting a conditioned aversion perhaps due to a general toxic effect, a common occurrence in rodents. At a low dose the title compound (shortened to "indole imidazoline") has been found to produce aversion to ethanol which promptly reverses upon withdrawal of the drug. Total fluid consumption is unaffected in a two choice situation, indicating that ethanol aversion is not a general toxic effect in this case.

Materials and Methods. Twenty-five male mice of the C57BL/6j strain (Jackson Lab., Bar Harbour, ME) were used in the investigation because of their known high self-selection index for alcohol (5). The animals were given a choice between distilled H_2O and a 10% solution made from 95% ethanol and distilled H_2O. The substances were presented in 15 ml centrifuge tubes graduated in 0.1 ml increments. Readings were taken every 24 hr for 10 days predrug, 4 days during-drug treatment and 4 days postdrug. Every other day the position of the bottles was switched to control for position effects. During drug treatment days, indole imidazoline as the tosylate salt was placed in both the water and 10% ethanol so that the mice would obtain 5 mg/kg/day of the salt based on a consumption level of 5 ml/day. These values were selected as optimal after pilot experiments.

Blood levels of ethanol and acetaldehyde following intraperitoneal injection of ethanol or ethanol plus indole imidazoline were obtained. Mice were injected intraperitoneally with a 10% solution of redistilled ethanol (1 g/kg) in physiological saline. Half the animals received 1 mg/kg indole imidazoline administered subcutaneously in the back immediately after injection of ethanol. A single solution of ethanol was prepared, sealed with a serum bottle cap, stored at 4°, and used for the entire experiment. Analytically, there was no change in concentration from beginning to end of the study.

At appropriate times the mice were sac-

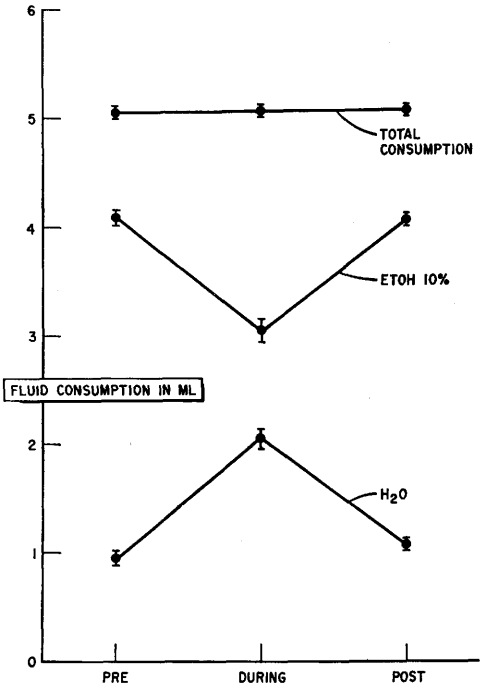

FIG. 1. Mean consumption levels by C57BL/6j mice for 4 days predrug, 4 days during-drug, and 4 days postdrug. Vertical lines indicate SE.

[1] This research was completed while C. W. S. was an employee of The Dow Chemical Company.

rificed by cervical dislocation, the thorax was opened and blood was withdrawn from the right ventricle directly into an heparinized chilled Hamilton 100 μl syringe. Samples were analyzed in duplicate by a slight modification of the method of Roach and Creaven (7). Following addition of the blood to serum vials containing 50 μl of 5% zinc sulfate and 50 μl of an internal standard solution of isopropanol (2.5 mg/ml), 50 μl of 0.3 *N* barium hydroxide was injected, the sample was mixed briefly on a vibratory mixer and centrifuged at 3100 rpm for 30 min at 4°. The clear supernatant was removed with a Hamilton syringe and 1 μl was injected into a Varian Aerograph (Model 2100) gas chromatograph.[2] The results are shown in Figs. 2 and 3.

Results and Discussion. Figure 1 illustrates the change in consumption during drug treatment. While total consumption remained essentially unchanged, ethanol consumption decreased by slightly more than 1 ml and water consumption showed a reciprocal increase. Statistical tests of the differences between the pre- and during-drug values yielded $t = 5.82$, $df = 24$, $p < .0005$ for decrease in ethanol and $t = 6.10$, $df = 24$, $p < .0005$ for the increase in water.

The self-selection indices (ml ethanol solution consumed/ml water and ethanol solution consumed) for the pre-, during-, and postdrug days were 0.82, 0.59, and 0.77, respectively. Within 24 hr after treatment began the index dropped to 0.56, and remained fairly stable at the new level. When the drug was withdrawn the index began to approach the predrug level immediately, reaching 0.71 during the first 24 hr. A *t* test of the differences between the pre- and during-drug indices yielded $t = 6.47$, $df = 24$, $p < .0005$.

The rate of disappearance of acetaldehyde in the drug-treated group does not appear to differ enough from the nontreated group to account for the differences in self-selection. On the other hand, the rate of disappearance

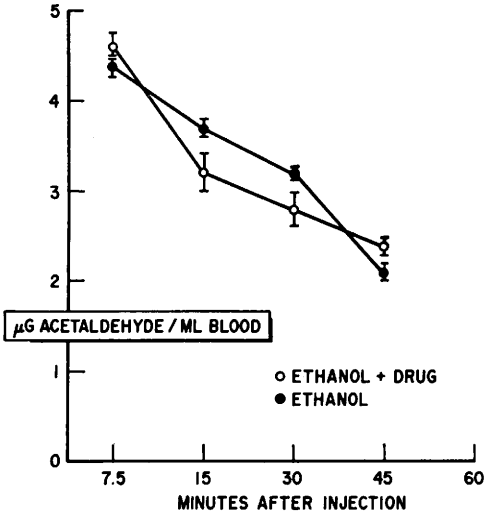

FIG. 2. Mean levels of blood acetaldehyde in C57BL/6j mice for drug treated and ethanol alone. Each point is a mean of 12 determinations, and the vertical lines indicate SE.

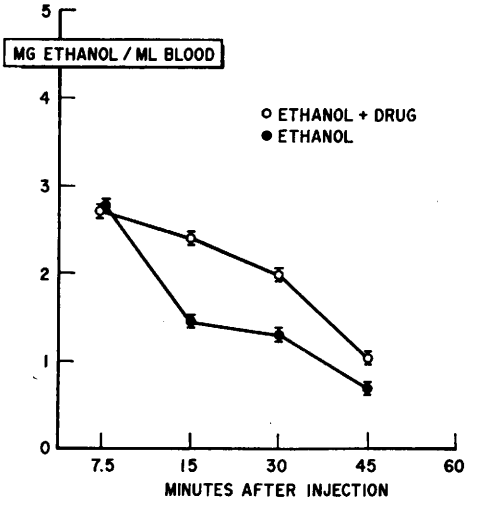

FIG. 3. Mean levels of blood ethanol in C57BL/6j mice for drug treated and ethanol alone. Each point is a mean of 12 determinations and the vertical lines indicate SE.

[2] Instrument: Varian Aerograph Model No. 2100 gas chromatograph. Two columns, two flame detectors. Column: Two 316 in. o.d. × 10 ft. stainless steel filled with Poropak Q 50-80. Conditions: Oven temperature, 120 ± 0.2°; injector, 200°; detector, 200°; carrier, N_2;

	Col. A	Col. B
Inlet pressure (lb)	26	24.5
Flowmeter reading	40	40
ml/min	90	90

Detector sensitivity, 10^{-12}; Detector attenuation 128 to 4 (as required for peak size); special injection port packed with Chromosorb WAW 60-80.

of ethanol appears to be slowed in the drug-treated group. The pattern appears to be similar to that induced by pyrazole (6). Rogers (5) has suggested that mice will not drink more ethanol in a two choice situation than they can metabolize or eliminate. If this is indeed the case, then the mice may reduce their consumption into line with the lower rate of ethanol removal that occurs when the solution drunk contains 3-[(2-imidazoline-2yl)methyl] indole. Although it does not seem to offer a clinically useful approach, the drug itself appears to be a helpful addition to those now available for research in this field.

Summary. Self-selection indices of a 10% ethanol solution were obtained from male mice of the C57BL/6j strain in a two choice situation prior to treatment with indole imidazoline. During treatment the drug was placed in both the ethanol solution and water. This resulted in an immediate and significant decrease in ethanol consumption. Self-selection indices returned to predrug levels within 24 hr after removal of the drug.

Gas chromatographic analysis of blood levels of ethanol and acetaldehyde suggested that the rate of metabolism of ethanol was slowed after the injection of the drug.

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