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9. M. Lieberman, *Circ. Res.* **21**, 879 (1967). Hearts from chick embryos, ages 11 to 13 days, were excised aseptically in saline G, placed in a petri dish, and minced into small fragments (smaller than 0.5 mm.) The fragments were transferred to a 25-ml Erlenmeyer flask containing 10 ml of warmed 0.1 percent trypsin in saline G and gently agitated in a shaker bath at 37°C. After 5 minutes the suspension was triturated slowly (five passes through a 5-ml pipette) and returned to the shaker bath for a 5-minute period of agitation. The cell suspension with trypsin was then transferred to 20 ml of cold culture medium, and the dissociation procedure was completed by slowly pipetting the suspension ten times. After being filtered through multiple layers of sterile gauze, the suspension was centrifuged for 10 minutes at 1200 rev/min. After the supernatant was discarded, the pellet was resuspended in 2 ml of culture medium. A cell count was made with a hemocytometer, and the suspension was rediluted as necessary to obtain concentrations of 10^5 and 10^6 cell/dish. The cultures were incubated from 6 to 21 days in a humidified chamber at 37°C with a continuously freshened atmosphere of 5 percent CO_2 and 95 percent air. Fresh media was added after 3 to 5 days, and the total volume in the dish was brought to 5 ml or less. Subsequently, the media was replaced every 3 to 5 days.
10. Materials were obtained as follows: trypsin 1-300, Nutritional Biochemical Corp.; tissue culture dishes No. 3002, Falcon Plastics; Eagle's minimum essential medium, North American Biological Inc.; medium 199 and selected lots of fetal calf serum, Grand Island Biological Co.; penicillin G and streptomycin sulfate, E. R. Squibb Inc.
11. Dissociated chick heart cells (2×10^6 cells per 100-mm dish) were seeded in 10 ml of fresh culture medium. After 2 days, the medium was changed and 20 ml of fresh medium was added. The medium was then collected after 4 days and centrifuged for 20 minutes at 1200 rev/min, and the supernatant was filtered through 0.45 μm Millipore filters and stored at 4°C.
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14. Ionic concentrations of all media were determined with a Coleman model 21 flame photometer and were as follows: Na^+, 140 meq/liter; K^+, 5.6 meq/liter; and Ca^{2+}, 3.2 meq/liter.
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18. We thank J. Mailen Kootsey for help in development of the electrophysiological procedures, and E. Clark and O. Oakeley for technical assistance. Supported in part by NIH grants T01-GM00929 and HE 12157; American Heart Association grant 71160; and the North Carolina Heart Association. This work was done in part during the tenure of an established investigatorship of the American Heart Association to M.L.

7 September 1971; revised 29 November 1971 ■

Δ^9-Tetrahydrocannabinol: Aversive Effects in Rat at High Doses

Abstract. *Water-deprived rats were administered a single dose of Δ^9-tetrahydrocannabinol either orally or intraperitoneally immediately after their first taste of a saccharine solution. In tests beginning 47 hours after drug administration, a dose-related reversal of rats' normal preference for saccharine was found. The data suggest that the drug produces aversive effects at doses of 1 to 32 milligrams per kilogram.*

Several investigators (*1*) have found a decrease in food intake by animals treated with Δ^9-tetrahydrocannabinol (Δ^9-THC), the principal active constituent of marihuana (*2*). Perhaps the clearest demonstration of this phenomenon was made by Manning *et al.*, who showed that Δ^9-THC produced a decrease in food consumption over a 30-day chronic administration period (*3*). This finding contradicts both a body of folklore concerning the effects of marihuana on human appetite for food and a recent study by Hollister (*4*), who showed that about half the human subjects given oral doses of Δ^9-THC increased food intake. A possible explanation of the discrepancy lies in differences in doses given to human and animal subjects, for animal subjects are typically given doses 10 to 100 times larger than human subjects are given. Studies in which high doses of THC are given to humans report that these doses may be slightly toxic in the sense that they produce unpleasant somatic effects (*5*). The toxicity of Δ^9-THC is also suggested by animal studies in which cats given doses from 0.5 to 4.0 mg/kg vomited and monkeys given doses from 16 to 64 mg/kg died (*6*).

We have used the "bait shyness" phenomenon to assess possible aversive effects of Δ^9-THC. "Bait shyness" or conditioned food aversion occurs when an animal ingests some food, usually a novel-tasting one, and then gets sick within the next 8 hours or so. In the laboratory, this phenomenon has been demonstrated for a variety of different tastes and various types of illness-inducing agents, including x-irradiation, injections of drugs such as apomorphine, methamphetamine, and various anesthetics. These studies have shown that rats will avoid novel-tasting substances that are followed by illness, even when the illness occurs as much as 8 hours after ingestion of the substance; the amount of aversion is related to the degree of illness (*7*).

Water-deprived rats were given various doses of Δ^9-THC from 0 to 32 mg/kg immediately after a period of exposure to a saccharine solution. They were then given preference tests between saccharine solution and tap water each day for 3 days, beginning 47 hours after drug administration. Saccharine preference was found to be inversely related to dose of Δ^9-THC, strengthening the view that THC may be aversive in high doses.

Forty experimentally naive male albino rats from the Walter Reed colony served as subjects (*8*). All weighed between 200 and 250 g at the start of the experiment and were singly housed in metal metabolism cages. The animals had continuous access to 45-mg rat food pellets (Noyes). After 2 days of free access to food and water, the animals were changed to a 23-hour water deprivation schedule in which tap water was available for 1 hour a day from a standard drinking tube. On the fourth day of this restricted drinking schedule, a 0.1 percent solution of saccharine in tap water, rather than pure tap water, was made available during the watering period. Immediately after this exposure, various doses of Δ^9-THC were administered either directly into the stomach with an oral administration needle or by intraperitoneal injection (*9*). The drug was diluted to concentrations of 2.25, 9.0, and 36.0 mg/ml with propylene glycol for administration to three groups of 10 animals each, with half the animals of each group getting oral administration and the other half, intraperitoneal administration. These concentrations were selected so that the dose volume would be approximately 0.2 ml when doses of 2, 8, and 32 mg/kg were given to the three groups. A fourth group of 10 animals received a placebo dose consisting of propylene glycol containing ethanol in the same proportion as the 36.0 mg/ml propyl-

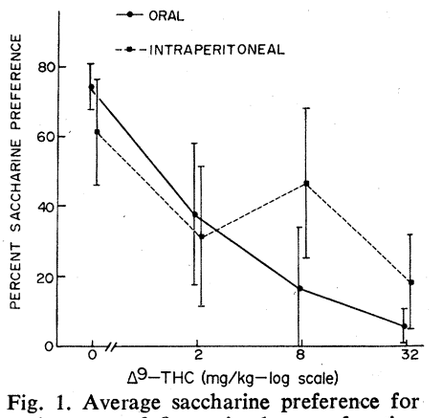

Fig. 1. Average saccharine preference for each group of five animals as a function of the dose of Δ^9-THC received by that group. Preference was computed as 100 times the amount of saccharine consumed divided by the total fluid intake, and was averaged across the three performance tests for each animal. The vertical lines show standard deviations.

ene glycol–THC solution, again with half receiving each route of administration.

On the day after drug administration, tap water only was made available to the animals during the watering period. On the second, third, and fourth days, two drinking tubes were made available to the animals during the watering period, one containing tap water and the other saccharine solution. The amount drunk from each tube was determined by weighing the drinking bottles before and after the watering period. The position of the two tubes was switched each day. Throughout the experiment the amount of food eaten each day was determined by weighing the food containers immediately before the watering period.

The data were broken down by route of administration, since considerable differences in data may have been produced by the different absorption routes.

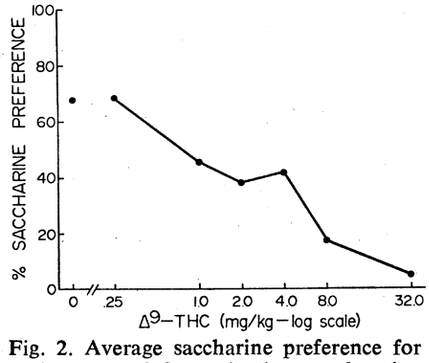

Fig. 2. Average saccharine preference for each group of five animals as a function of the oral dose of Δ^9-THC received by that group.

In fact, the data from the two routes of administration were quite similar with the exception of two animals in the 8 mg/kg intraperitoneal group which showed no effects, presumably because the drug was injected into the lumen of the intestine. As reported in other studies, there was a dose-related decrease in food intake in both the oral and intraperitoneal groups during the first 24 hours. A preference score for each animal was computed each day by dividing the weight of saccharine solution consumed by the weight of the total amount of fluid consumed and multiplying by 100. Thus, 100 indicates that all the fluid consumed was saccharine, and a zero indicates that no saccharine was consumed. Figure 1 shows the mean and standard deviation of preference scores averaged across all three preference tests. Even if the data from the two errant animals is retained, an analysis of variance showed that there was a significant effect of dose level of THC on saccharine preference for both routes of administration, though the effect for the oral route was greater [$P < .001$, $F = 16.95$ (d.f. = 3,16)] than for the intraperitoneal route [$P < .05$, $F = 3.70$ (d.f. = 3,16)]. Even at the lowest dose level, the effect was significant as determined by t-tests comparing the placebo groups with the 2 mg/kg groups [oral: $P < .005$, $t = 3.36$ (d.f. = 8); intraperitoneal: $P < .025$, $t = 2.38$ (d.f. = 8)]. Thus, animals that received only a placebo after their first exposure to a saccharine solution showed the normal preference for saccharine, while the drug-treated animals demonstrated a reversal of this preference.

To extend the dose-response curve, and to examine the possibility that at doses lower than 2 mg/kg the Δ^9-THC would not have any aversive effects, or even that, relative to placebo controls, an increase in preference might be found, four additional groups of five animals each were run. Three of these groups were drug groups, receiving Δ^9-THC in oral doses of 0.25, 1.0, or 4.0 mg/kg immediately after their first exposure to the saccharine solution. The fourth group received a placebo. The mean preference scores for the three drug groups were, respectively, 69.1, 45.0, and 41.4 percent, with the placebo group scoring 59.5 percent. In Fig. 2, these data are plotted together with the oral group means from the original experiment. The placebo point represents the average of the two oral placebo groups. These additional data show that there is in fact a consistent dose-related aversion effect, and that there is no reversal of the effect at low doses.

To the extent that the procedures of the present study may be said to constitute a measure of aversiveness, the higher doses of Δ^9-THC administered in this study clearly were aversive. The aversion was not related to route of administration, and it was related to dose. Thus, inconsistencies between observations of increased hunger in human marihuana users and decreased food intake in animals given Δ^9-THC may lie in the fact that the high doses of Δ^9-THC typically given to animals make them sick and thereby decrease their intake of food. The finding that high doses of Δ^9-THC are aversive is particularly important in view of the fact that virtually all animal studies of marihuana use doses in this range. Our experiment suggests that such experiments are studying toxic effects of Δ^9-THC and may be of minimal value in elucidating the subtle and elusive psychological changes reported to occur in marihuana intoxication.

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9. The Δ^9-THC was supplied by J. A. Scigliano of the Center for Studies of Narcotic and Drug Abuse of NIHM. It was in ethanol solution in a concentration of 0.2 g/ml when received, and was assayed to be 93 percent pure.

8 November 1971