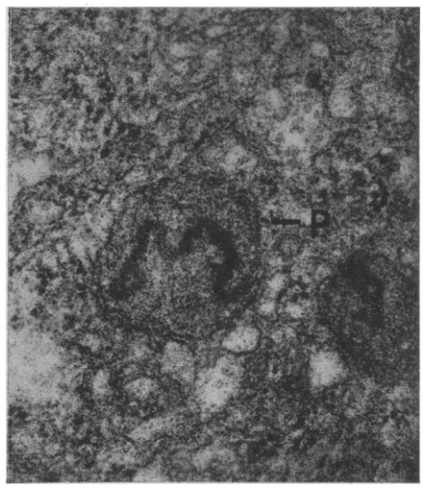

Fig. 3. Typical peroxisome derived from hepatic tissue of the frog, exhibiting a subcrystalloid core in the form of a complex tubular inclusion. Tissue fixed in glutaraldehyde and postfixed in osmium, followed by staining with uranyl acetate and lead citrate (× 47,000).

oxidase was 1.266. Particles with which urate oxidase and α-hydroxyacid oxidase were associated displayed a medium density of 1.278. These values were in contrast to median densities of 1.183 for particles containing acid phosphatase and 1.229 for particles containing succinic dehydrogenase. Thus, particles containing allantoinase resemble peroxisomes rather than mitochondria or lysosomes in their density distribution.

The disparate denstiy distribution shown by particles containing allantoinase, catalase, and D-amino-acid oxidase, when compared to those containing urate oxidase and α-hydroxyacid oxidase, is puzzling. The association of urate oxidase with the crystalloid core of peroxisomes has been advanced as an explanation of the similar behavior of these particles when derived from rat liver (*8*). It is possible, in frog liver, that both urate oxidase and α-hydroxyacid oxidase are associated with the peroxisome core. Peroxisomes from this source show a well-developed subcrystalloid core (Fig. 3) that often appears as a complex tubular inclusion exhibiting lateral extensions. They are closely associated with the endoplasmic reticulum, and occasionally the membrane of the particle appears as a direct saclike extension of the latter.

The presence of allantoinase in peroxisomes of amphibian liver suggests that these particles participate in the degradation of uric acid to glyoxylic acid and urea as well as in the removal of hydrogen peroxide by catalatic oxidation. Our investigations indicate that allantoincase, the enzyme acting in the cleavage of allantoic acid, is associated primarily with the soluble fraction derived from hepatic tissue of the adult frog.

Evidence indicates that peroxisomes of mammals are responsive to hypolipedemic agents (*9*) as well as to irradiation (*10*). This evidence and our data raise the question of the role of the peroxisome in cells of animals undergoing natural environmental pressures and fluctuating life cycles. In plants and *Tetrahymena* the peroxisome has been identified as the site of gluconeogenesis from 2-carbon compounds by virtue of its content of glyoxylate cycle enzymes (*5*). The role of the peroxisome in any unified metabolic scheme in mammals is obscure. In these animals peroxisomes have been described as "fossil organelles" (*8*), serving as important sites of peroxide catabolism and extramitochondrial oxidations. The association of allantoinase with amphibian peroxisomes and the position of this enzyme in the uricolytic sequence leading to glyoxylic acid and urea suggest that the peroxisome, at least in more primitive organisms, may play a role in uric acid catabolism.

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1-Δ⁹-Tetrahydrocannabinol: Neurochemical and Behavioral Effects in the Mouse

Abstract. *Administration of pure 1-Δ⁹-tetrahydrocannabinol to mice had the following dose-dependent neurochemical and behavioral effects: a slight but significant increase in concentrations of 5-hydroxytryptamine in whole brain; a decrease in concentration of norepinephrine in brain after administration of low doses and an increase after high doses; diminished spontaneous activity, moderate hypothermia, hypersensitivity to tactile and auditory stimuli, and ataxia after low doses; and sedation, pronounced hypothermia, and markedly diminished spontaneous activity and reactivity after high doses. The duration of the effects on body temperature and spontaneous activity correlated generally with the changes in brain amines. The characteristic changes in brain amines do not correspond exactly to those observed with other psychotropic drugs.*

The availability (*1*) of pure, synthetic 1-Δ⁹-tetrahydrocannabinol (Δ⁹-THC) has permitted study of its effects both on amine metabolism in the brain and on gross behavior in the mouse. In the past, study of the psychopharmacological effects of marihuana has been complicated by the fact that extracts in different laboratories contain varing mixtures of tetrahydrocannabinol isomers, along with other constituents. The effects of low doses (50 to 120 μg) of Δ⁹-THC per kilogram of body weight in man (*2*) have been described as similar to those of marihuana; that is, euphoria, alterations in sense of time

and in visual and auditory perception, slight tachycardia and terminal sedation are often reported; higher doses (200 to 480 μg/kg) produced auditory and visual hallucinations, agitation, increased tachycardia, personality changes, and sedation. The behavioral classification of a compound with euphoriant, psychotomimetic, and sedative properties is difficult (*3*). We focused on the psychopharmacological and biochemical effects of Δ^9-THC because of the observation of its dose-dependent effects in man.

Synthetic Δ^9-THC was received in ethanol solution in sealed glass vials under nitrogen. It was stored as such at −10°C and protected from light (*4*) until ready for use. Solutions suitable for injection were prepared by evaporation of ethanol and suspending the Δ^9-THC residue in saline and Tween-80 (three to six drops). Solutions were made up 18 hours (or less) before injection, kept at −10°C, and protected from light. Thin-layer chromatograms of such suspensions showed only the Δ^9-THC spot. All experimental animals received an intraperitoneal injection (0.5 ml) of the drug suspension at room temperature; all control animals received an equal volume of the saline-Tween vehicle. Tween-80 did not significantly affect the brain amines.

Female mice (C57B1/6J), 5 to 6 weeks old (14 to 20 g), (Jackson Laboratory, Bar Harbor, Maine) were used. After receiving injections, the drug and control animals were caged separately, six animals to a cage. At the appropriate time, the animals were decapitated, and the brains were removed and homogenized in 0.01*N* HCl. Norepinephrine (NE) and 5-hydroxytryptamine (5-HT) were extracted from the homogenates, and the concentrations of amines were measured spectrophotofluorometrically (*5*). In other experiments, 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) were extracted from three pooled mouse brains, and the amounts of each were determined in separate samples by a modification (*6*) of the methods of Bogdanski *et al.* (*7*) and of Udenfriend *et al.* (*8*). Standard solutions of known concentration of each amine were carried through the entire assay procedure. The recovery of added amines and 5-HIAA was determined with brains from both treated and control animals. The presence of Δ^9-THC in the solutions did not interfere with the fluorometric assay of either of the amines or of 5-HIAA. Each set of experimental animals was studied concurrently with control animals. Means were calculated for each dose and time. The differences in mean amine concentrations are expressed as a percentage of control values.

Doses of Δ^9-THC greater than 5 mg/kg increased the concentration of 5-HT in whole brain, with significant elevations at 10, 100, 200, and 500 mg/kg. Doses of 5 to 10 mg/kg significantly decreased NE concentrations, whereas doses of 200 and 500 mg/kg significantly increased them (Fig. 1).

The time course of the changes in 5-HT and NE was determined at Δ^9-THC doses of 10 and 200 mg/kg (Figs. 2 and 3). At the lower dose 5-HT increases to a maximum at 45 minutes and returns to control values between 3 and 6 hours (Fig. 2). After administration of 200 mg/kg, the elevation of 5-HT lasts somewhat longer, and occurs also at 24 hours (Fig. 3). The NE decrease after administration of 10 mg/kg lasts 3 to 6 hours. The elevation in NE after administration of 200 mg/kg is not evident until 45 minutes and also is seen at 24 hours. The elevation of NE at 200 mg/kg thus parallels closely the duration of the elevation of 5-HT. Although slightly elevated, 5-HIAA concentrations did not show statistically significant changes with either dose or time; it is therefore probable that there is no inhibition of monoamine oxidase in vivo.

All the animals receiving Δ^9-THC in doses greater than 1 mg/kg had consistent hypothermia. At a dose of 10 mg/kg, body temperature decreased 2° to 3°C below that of the control animals within 30 minutes; normal temperature was regained within 2 to 3 hours. At 50 mg/kg, temperature decreased by 3° to 5°C. All animals which received 200 mg/kg or more were hypothermic within 5 to 10 minutes. The hypothermia lasted at least 24 hours; by 48 hours body temperature was normal. At a dose of 500 mg/kg, the decrease in body temperature was 5° to 6°C. Thus the onset of the hypothermia was rapid, and its duration roughly paralleled the period of altered concentration of amines.

With lower doses (1 to 10 mg/kg) of Δ^9-THC, many animals appeared ataxic and hyperreactive to auditory and tactile stimuli, but they still showed decreased spontaneous activity, recovering in 3 to 4 hours. After administra-

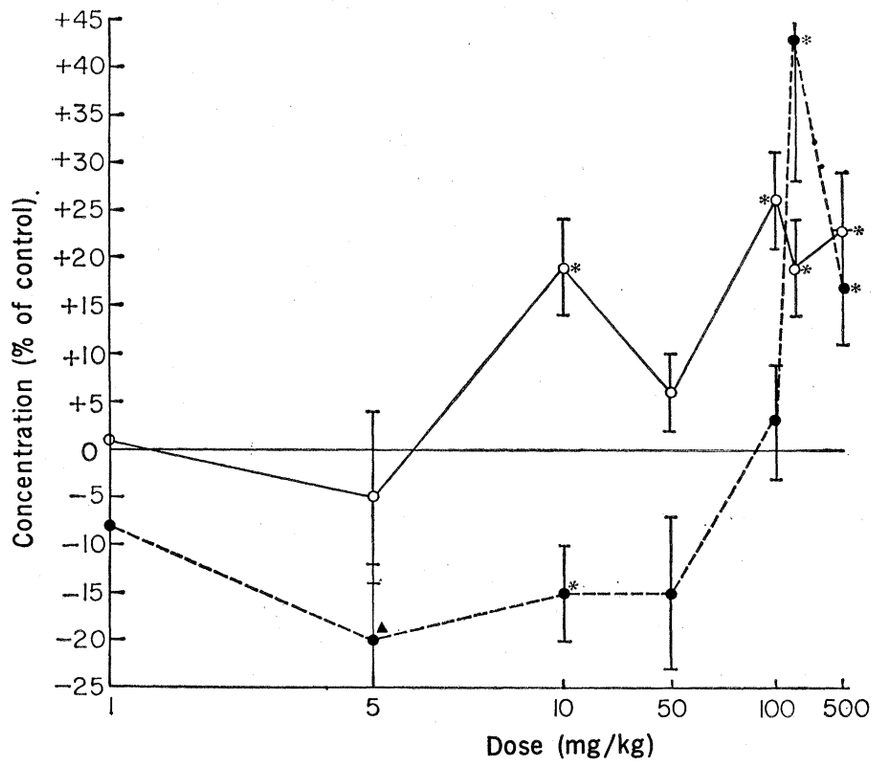

Fig. 1. Dose effects of Δ^9-THC on concentrations of 5-HT (○———○) and NE (●-------●) in whole brain 45 minutes after injection. Each point represents the difference in the mean value of at least 11 experimental animals from the mean value of concurrently run control animals, expressed as a percentage of that control value. Control animals received an equal volume of the vehicle which consisted of saline and Tween-80. Vertical bars indicate standard error. Significant differences from control means are indicated by a solid triangle ($P < .02$) and by asterisks ($P < .01$).

tion of 10 mg/kg, the peak hypoactivity was observed at 90 minutes, when spontaneous activity was 50 percent that of controls, as measured in a Lehigh activity cage. After receiving 200 mg/kg, the mice appeared highly sedated within 5 minutes, and their spontaneous activity was 80 percent that of controls at 30 minutes, with recovery at 24 to 36 hours. These animals were only briefly aroused by prodding.

Scheckel *et al.* (*9*) studied the effects of the Δ^8- and the Δ^9-isomers of tetrahydrocannabinol on monkeys and reported that both produce marked behavioral changes—stimulation (or depression, depending on the dose), apparent hallucinations, and loss of ability or motivation to perform complex tasks. Some of the behavioral effects of Δ^9-THC here reported have been observed before in rodents with marihuana extracts. Miras observed that in the rat doses of 200 to 250 mg/kg injected intraperitoneally produced hypothermia which was maximum at 2 hours, normal temperatures being regained at 6 hours (*10*). With similar extracts, Garattini found that a moderate degree of hypothermia and decreased locomotor activity occurred after doses of 50 to 200 mg/kg to mice and rats; the stimulation induced by amphetamine in mice or by tryptamine in rats was not prevented (*11*). The effect of barbiturates was potentiated, but alterations of the brain amines at 45 minutes, 2 and 4 hours after administration of 150 to 300 mg per kilogram of body weight intraperitoneally or 2.5 mg intracerebrally were not consistent (*11*). Dagirmanjian and Boyd had earlier found that the barbiturate sleeping time (the time between the loss and the regaining of the righting reflex after intraperitoneal administration of 100 mg of sodium hexobarbital per kilogram of body weight) of mice and the amphetamine-induced increase in activity were prolonged by two tetrahydrocannabinol derivatives dimethylheptylpyran and methyloctylpyran (*12*).

The duration of the hypothermia and of the behavioral changes in mice is generally correlated with the duration of the changes in amine concentrations in brain. The dose range over which a reduction of NE was observed is the same as that in which the transient periods of hyperreactivity and ataxia were seen. Whether the changes in amine concentration in the brain and the hypothermia are causally related is not apparent. Little is known of the uptake of Δ^9-THC in the brain and its metabolism.

The relation of Δ^9-THC to other psychoactive drugs is of interest. Amphetamine produces hyperreactivity and also lowers NE and raises 5-HT concentrations in the brain (*13*), but spontaneous activity is increased, unlike the response to Δ^9-THC. Animals which receive lysergic acid diethylamide (LSD) are hyperreactive to stimuli and also show decreases in brain NE and increases of brain 5-HT (*14*) of the same order of magnitude observed with Δ^9-THC or high doses of amphetamine. However, after administration of LSD, brain 5-HIAA is decreased, which in-

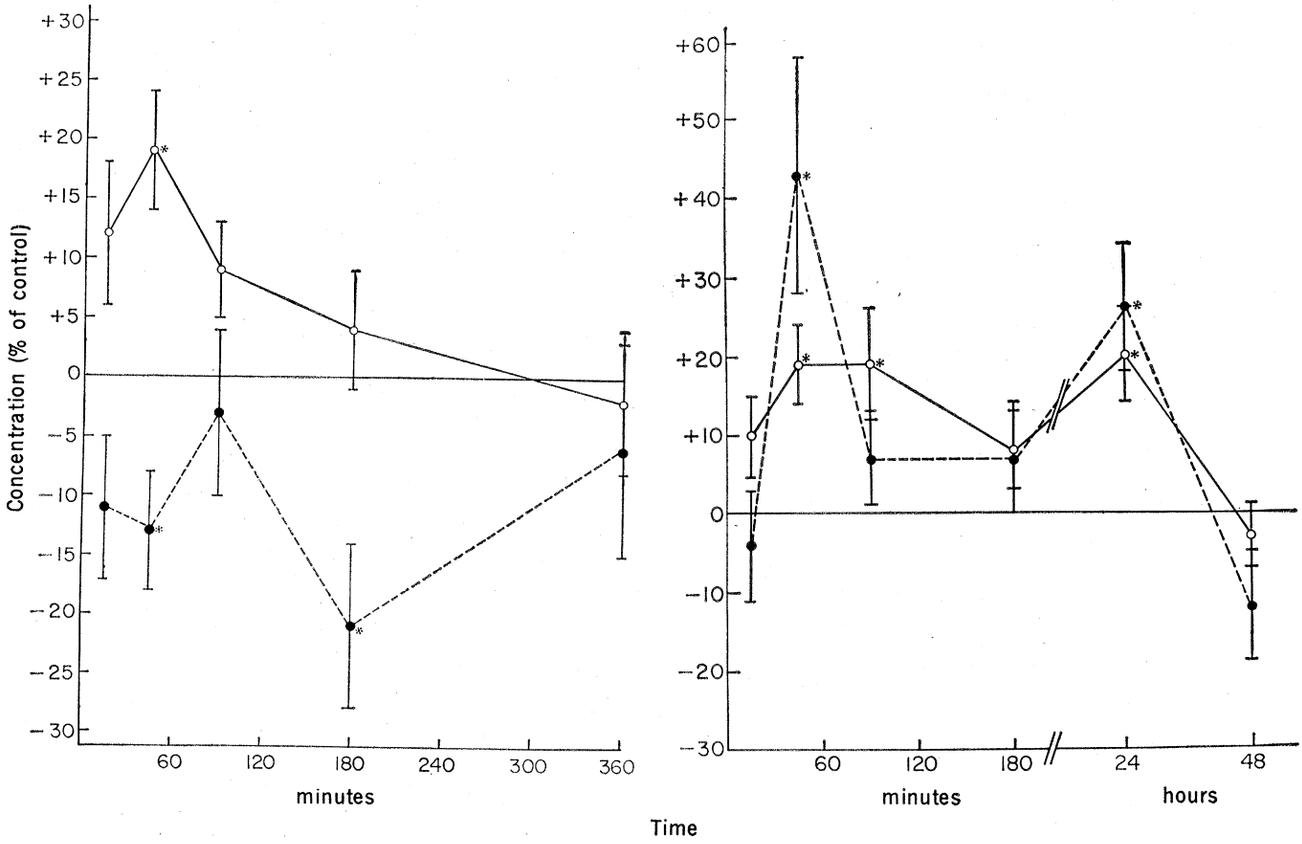

Fig. 2 (left). Time course of the effects of Δ^9-THC, at a dose of 10 mg/kg, on concentrations of 5-HT (○———○) and NE (●-------●) in whole brain. Symbols and procedures as in Fig. 1. Significant differences from control means ($P < .01$) are indicated by an asterisk. Fig. 3 (right). Time course of the effeects of Δ^9-THC, at a dose of 200 mg per kilogram of body weight, on the concentrations of 5-HT and NE in whole brain. Symbols and procedures as in Figs. 1 and 2. Significant differences from control means ($P < .01$ are indicated by an asterisk.

dicates a decreased metabolism or an increased "binding" of 5-HT (*6*). Determination of the effects of Δ^9-THC on more refined parameters of amine metabolism, such as turnover, uptake at nerve endings, and localization in critical regional or subcellular compartments, is obviously required to differentiate the various psychoactive drugs which influence brain amine metabolism. For instance, the 5-HT changes should not be interpreted as a specific effect upon 5-HT receptors. The changes reported, although reliable (*15*) and significant, are quite variable and could conceivably be a pharmacologically nonspecific effect and due to stress or stimulation (*16*). Our results indicate that the Δ^9-isomer of tetrahydrocannabinol does produce many of the effects of marihuana in animals, as it does in humans.

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Alcohol and Amitriptyline Effects on Skills Related to Driving Behavior

Abstract. *Three motor-skill tests related to driving ability were given to 21 healthy young volunteers after administration of various combinations of amitriptyline, placebo, and alcohol. It was found that the tricylic antidepressant added to the deleterious effects of alcohol.*

Drugs which act on the central nervous system are being ever more widely prescribed (*1*), and it has been shown that chlorpromazine has a supplementary and possibly potentiating effect on the impairment of coordination and judgment produced by alcohol (*2*). Although an initial moderate dose of either chlordiazepoxide or meprobamate does not significantly potentiate the effects of alcohol (*3*), there is still little information available concerning the interaction of most prescribed psychotropic drugs with alcohol. Since animal studies indicate that antidepressants may add to the effects of alcohol (*4*), the effects of amitriptyline (a commonly prescribed antidepressant) and alcohol on some skills related to driving behavior were tested in humans.

Healthy medical students (18 men and 3 women; mean age 22.14 years, S.D. = 1.15) volunteered as subjects. Full blood counts and liver function tests were performed on each subject before and after the experiment; no subject on any medication or with a history of recent illness or liver disease was accepted. They were cautioned against drinking and driving for several days after.

The subjects were randomly placed in one of three groups of seven subjects each; however, one woman was allocated to each group. Group A received amitriptyline twice; the first dose on the night before and the second on the morning of the day on which the tests were administered (interval, 12 to 15 hours). Group B received placebo at night and amitriptyline on the test day. Group C received placebo tablets on both occasions. A double-blind technique was used for drug administration and for the recording and scoring of test results. Amitriptyline (0.8 mg per kilogram of body weight) was given in tablet form for each dose.

After a medical examination on the morning of the test day, the subjects received their second issue of tablets, and 2 hours later they were tested with three motor-skill tests given in random order.

The simulated driving task was a modification of a test designed by Gibbs (*5*). The subject was seated before a steering wheel and required to move a pointer to a position in line with one of five horizontal lights placed at eye level. These lights flash on in a random order for 1.27 seconds, and the pointer has to be steered from one light to another as they go on in turn. This tedious and repetitive task is designed to show up the effects of fatigue. Only errors were scored, that is, movements in the wrong direction, omissions, and inadequate or otherwise incorrect steering of the pointer. The test lasted 12 minutes, but only the last 150 seconds were scored. Pen-recording equipment and the observer were based in a room separated from the subject by a one-way screen.

The dot-tracking test required a continuous line to be drawn between small dots that were arranged in an irregular