Acute Toxicity of Δ^9-Tetrahydrocannabinol in Rats and Mice[1] (35241)

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(Introduced by J. Ashmore)

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Cannabis sativa (marihuana) is among the oldest drugs known to man. Its intoxicating properties have placed it in the forefront of contemporary drug abuse, although little is known concerning its ultimate effects. Intensive research programs have been initiated in recent years in an attempt to isolate its active components, define its effects, and elucidate its mechanism(s) of action.

Many of the chemical components present in marihuana have been determined. Adams *et al.* (1) ascertained the structures of cannabinol and cannabidiol. Gaoni and Mechoulam (2) isolated Δ^9-tetrahydrocannabinol and Jen *et al.* (3), have successfully synthesized Δ^8-tetrahydrocannabinol. Numerous investigators (2, 4, 5) have shown Δ^9-tetrahydrocannabinol (THC) to be one of the principle pharmacologically active components of cannabis. Mechoulam *et al.* (6) have recently reported that it is the only psychotomimetically active component.

In the literature very few studies concerning the acute toxicity of cannabis in laboratory animals can be found. There are no acute toxicity studies utilizing pure Δ^9-THC. One investigation reported by Loewe (7) was conducted with tetrahydrocannabinol acetate, the acetyl ester being prepared from Oriental cannabis resis (charas). The ester was administered as a suspension with dipropylene glycol into mice orally, subcutaneously, and intravenously. LD_{50} values of 21.66, 11.0, and 0.175 g/kg, respectively, were obtained. More recent acute toxicity data has been reported by Joachimoglu (8). He conducted a comparison of extracts of natural cannabis and cannabis sublimate obtained by combusting a cannabis cigarette into a glass tube. The extracts were dissolved in olive oil and injected intraperitoneally into mice. Utilizing cannabis obtained from three sources and of five different ages, LD_{50} values ranging from 0.3 to 2.5 g/kg for the natural product and 1.0 to 17.0 g/kg for the corresponding sublimates were reported.

Since cannabis is used extensively throughout the world and Δ^9-THC has been shown to be its principle pharmacologically active component, toxicity studies are greatly needed to determine short- and long-term effects from using this compound. The basic reason for a lack of pharmaco-toxicological studies with THC has been the unavailability of this compound in pure form. A method however, has been developed by Turk *et al.* (9) which is capable of producing 99+% pure Δ^9-THC in sufficient quantities suitable for investigation. Therefore this paper reports acute toxicity studies of Δ^9-tetrahydrocannabinol administered intravenously, intraperitoneally, and intragastrically in rats and mice.

Methods. Several grams of Δ^9-THC were extracted and purified from marihuana plant stock. Assay by nuclear magnetic resonance, mass spectroscopy, and gas–liquid chromatography indicated the purity of the compound to be 99+%.

The Δ^9-THC was prepared for the studies by diluting a stock suspension to achieve the desired concentrations in appropriate volumes. In the acute toxicity study utilizing

[1] Presented at the Ninth Annual Meeting of the Society of Toxicology, Altanta, Georgia, March 15-19, 1970. Supported by U.S. Public Health Service Grant No. GM-1089 and U.S. Public Health Service Grant No. MH-15864.

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mice injected intraperitoneally, for example, 1.90 g of Δ^9-THC was weighed into a 50-ml conical graduated centrifuge tube. To this was added 3.93 ml of Tween 80 (polyoxyethylene sorbitan mono-oleate) and the mixture was shaken vigorously on a vortex shaker while heating the centrifuge tube with a stream of warm air. Isotonic saline was added dropwise to the mixture with continuation of the heating and shaking to make a total volume of 39.3 ml of homogeneous stock suspension. In order to inject 10 mice at a dose level of 400 mg/kg of Δ^9-THC and at a constant volume of 0.5 ml/25 g, 2.11 ml of stock suspension were added to 2.89 ml of isotonic saline to yield 5.0 ml of suspension for injection. The Δ^9-THC was prepared in a similar manner for all acute toxicity studies reported herein.

Before determining LD_{50} values, range finding doses were administered to two animals per dose level. The highest nonlethal dose in the range finding studies was used as the lowest dose for the LD_{50} determination. A geometric progression of 1.10 was begun from this lowest dose level according to the method of Weil (10) which was used to evaluate acute toxicity throughout this study.

Male albino Holtzman rats weighing beween 100 and 125 g were used in this investigation. They were divided into groups of two and placed in the animal care facility 24 hr prior to experimentation. All rats were maintained on Purina Laboratory Chow and tap water *ad libitum*. The rats which received intragastric doses were fasted for 24 hr following the 24-hr conditioning period, but were allowed tap water.

Male albino Cox mice weighing between 20 and 25 g were divided into groups of 10 for the intragastric and intraperitoneal studies and into groups of 6 for the intravenous study. They were placed in the animal care facility and handled in the same manner as described for the rats.

Following a 24-hr conditioning period and an additional 24 hr fast for the intragastric study, Δ^9-THC was injected intraperitoneally, intravenously, or intragastrically to 6 rats for each dose level. The volumes administered were 1.0, 0.25, and 1.0 ml/125 g of body weight, respectively. An equal number of control animals for each route of administration was also injected with a 10% Tween 80 in isotonic saline solution.

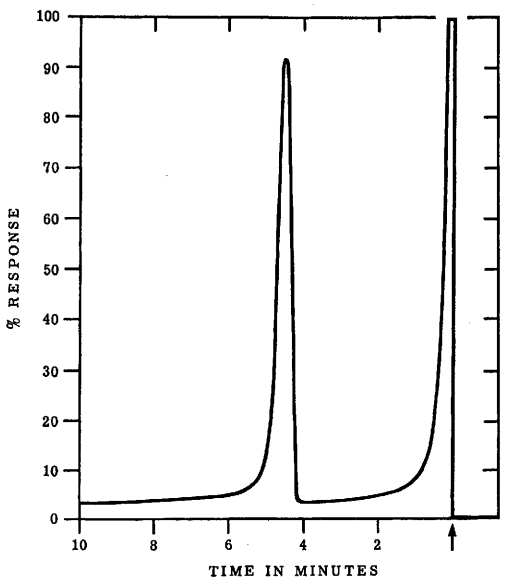

FIG. 1. Gas chromatogram of Δ^9-THC purified by column chromatography.

In the intraperitoneal and intragastric studies, Δ^9-THC was injected into 10 mice at each dose level following the 24-hr conditioning period and the fasting period in the case of intragastric administration. Injection volumes were 0.5 ml/25 g mouse. For the intravenous study, 6 mice per dose level were injected via the tail vein with a volume of 0.1 ml/25 g mouse.

Results and Discussion. Figure 1 represents a gas chromatogram of the Δ^9-tetrahydrocannabinol used throughout this study. This chromatogram was made after mixing Δ^9-THC with the 10% Tween 80 injection vehicle and shows the drug to be unaltered.

Table I shows the results obtained from the administration of Δ^9-tetrahydrocannabinol by the routes described for rats. When administered intravenously, the LD_{50} is 28.6 mg/kg with very narrow confidence limits. These limits are broader when the drug is given intraperitoneally, the LD_{50} value being 372.9 mg/kg. When administered intragastrically, an LD_{50} value of 666.1 mg/kg with relatively narrow confidence limits was obtained.

TABLE I. Acute Toxicity of Δ⁹-Tetrahydrocannabinol in Rats.[a]

Route of adm	No. of animals/ group	Observation time (days)	LD_{50}[b] (mg/kg)
iv	6	7	28.6 (27.4–29.85)[c]
ip	6	7	372.9 (304.8–453.7)
ig	6	7	666.1 (603.7–734.1)

[a] Vehicle: 10% Tween 80.
[b] Method of Weil.
[c] Confidence interval 95%.

After injection of Δ⁹-THC, the effects of the drug appear at various time intervals depending on the route of administration. When administered intravenously, the rats became ataxic within 1 or 2 min. However, upon stimulation by touch or sound, they became hyperexcitable for 1 or 2 sec in which they rushed about the cage. Following these initial signs, the animals became progressively depressed, although still somewhat responsive to external stimuli of sound or touch for approximately 1 hr. A continual low squealing was emitted by most rats for 1 to 2 hr. For those animals which died following intravenous injection of Δ⁹-THC, death always occurred within 15 min. Death was preceeded by the following signs: ataxia, hyperexcitability, depression, loss of righting reflex, and dyspnea progressing to apnea. Immediate post mortem examination of some rats revealed edematous and congested lungs at all dose levels. All toxic signs disappeared within 24 hr in the surviving animals.

Toxic signs associated with administration of the drug intraperitoneally and intragastrically were very similar to each other and in most respects to those following intravenous administration. Within 15 min post injection, either intraperitoneal or intragastric, all animals were ataxic and hypersensitive to touch or sound stimuli. Hyperexcitability, as noted for intravenous injection, was evident in all animals although to a lesser degree. Additional toxic signs which occurred in animals injected intraperitoneally or intragastrically were a continual tremor appearing approximately 4 hr post injection, diarrhea, and lacrimation. The tremor was more pronounced in the higher-dosed animals and would dissipate only after 2 to 3 days in surviving rats, although usually at least 3 days passed before all toxic signs disappeared. Death occurred between 10 and 36 hr and was preceded by the same signs as described for intravenous administration.

Table II shows the results obtained from the administration of Δ⁹-tetrahydrocannabinol to mice. From the data the mouse seems to be somewhat less sensitive to Δ⁹-THC than the rat following intravenous injection (LD_{50} of 42.47 for mice compared to 28.6 mg/kg for rats) and intraperitoneal injection (LD_{50} of 454.5 for mice compared to 372.9 mg/kg for rats). When given orally the rat appears to be less sensitive to the drug (LD_{50} of 666.1 mg/kg for rats compared to 481.4 mg/kg for mice).

Gross observations of mice postinjection support the findings of a lesser degree of toxicity following intravenous and intraperitoneal injection of Δ⁹-THC. The signs, however, are nearly indentical to those described for rats. There is an initial ataxia followed by hypersensitivity to external stimuli, depression, loss of righting reflex, and dyspnea progressing to apnea. Following intraperitoneal and intragastric injection, surviving mice appeared to recover 12 to 24 hr sooner than did rats, although death occurred between 10 and 36 hr. The mice did not exhibit tremor,

TABLE II. Acute Toxicity of Δ⁹-Tetrahydrocannabinol in Mice.[a]

Route of adm	No. of animals/ group	Observation time (days)	LD_{50}[b] (mg/kg)
iv	6	7	42.47 (36.74–48.86)[c]
ip	10	7	454.5 (419.03–493.1)
ig	10	7	481.9 (450.75–515.17)

[a] Vehicle: 10% Tween 80.
[b] Method of Weil.
[c] Confidence interval 95%.

although diarrhea was evident after intragastric and intraperitoneal administration. One difference noted with intravenous injection in mice was a Straub-tail effect. This did not occur after intraperitoneal or intragastric administration and was not apparent at all in rats. Following intravenous administration, death occurred usually within 5 min, as in the case of rats, and never after 15 min.

Other investigators have noted many of the toxic signs described here after administering extracts of cannabis to rats and mice. These extracts generally were from marihuana sources known to be relatively high in Δ^9-tetrahydrocannabinol, *i.e.*, from approximately 25–75% of the cannabinoids present. Vieira *et al.* (11) reported that the organic layer of hashish smoke extracts in doses of 50 mg/kg elicited excitation, increased sensitivity to sound, and general depression in albino rats. Garriott *et al.* (12) noted a general depression in mice following administration of 25 mg/kg of an extract of cannabis.

Bose *et al.* (13) reported that following the administration of a dose range of 15–30 mg/kg of cannabis extract to mongrel dogs, behavioral changes were quickly observed. These included an initial excitement, followed by increased activity and restlessness after which depression and ataxia ensued.

It is interesting that many of the signs evident in animals after administration of cannabis extracts of Δ^9-THC are very similar to those described by Walton (14) and others (15–17) after smoking a cannabis preparation. These signs included hypermotility, fibrillary muscle tremors, hypersensitivity to sound, and depression.

Summary. Crude Thailand marihuana was extracted by the method of Turk. Initial purity of the Δ^9-THC after extraction was 99+% as determined by nuclear magnetic resonance, mass spectroscopy, and gas–liquid chromatography. Purity of the compound prior to administration was unchanged as determined by gas–liquid chromatography. Using 10% Tween 80 as a suspension vehicle, LD_{50} values were determined in rats and mice. Values obtained were: rat, iv, 28.6 mg/kg; ip, 372.9 mg/kg; ig, 666.1 mg/kg; mouse, iv, 42.47 mg/kg; ip, 454.5 mg/kg; ig, 481.9 mg/kg. Toxic signs preceding death in both animal species included ataxia, hyperexcitability, depression, loss of righting reflex and dyspnea progressing to apnea. Following intravenous administration in rats or mice, death occurred within 15 min whereas following intraperitoneal or intragastric administration, death resulted between 10 and 36 hr. Tremor, diarrhea, and lacrimation were observed as additional toxic signs following ig and ip administration of Δ^9-THC in rats. Diarrhea was an additional toxic sign observed following ig and ip injections in mice and a Straub-tail was noted only after iv administration in mice.

We thank Mrs. Alice B. Richards and Mr. John Preston for valuable technical assistance rendered during this study.

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Received Sept. 8, 1970. P.S.E.B.M., 1971, Vol. 136.