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EFFECTS OF MARIHUANA IN LABORATORY ANIMALS AND IN MAN

E.A. CARLINI & I.G. KARNIOL
Departamento de Psicobiologia, Escola Paulista de Medicina,
Rua Botucatu, 862-04023 São Paulo, Brasil

P. F. RENAULT & C.R. SCHUSTER
Department of Psychiatry, University of Chicago, Chicago, Illinois, U.S.A.

1 The pharmacological potencies of the resins from three different samples of Brazilian marihuana (A, B and C) were determined through corneal areflexia in rabbits, decrease of spontaneous motor activity and induction of catatonia in mice, and decrease of rope climbing performance of rats.

2 The Δ^9-tetrahydrocannabinol (Δ^9THC) content of the marihuanas, measured by gas chromatography, was 0.82, 2.02 and 0.52%, respectively, for samples A, B and C. Approximately 2% cannabinol was present in samples A and B whereas the content of cannabidiol was approximately 0.1%.

3 The petroleum ether extraction of the samples A, B and C yielded, respectively, 12.06, 14.56 and 4.26% of resin.

4 In all animal tests resin B was nearly twice as active as resin A, whereas C was the weakest.

5 The smoke of the marihuana samples was inhaled by 33 human subjects, under a double-blind standardized procedure. Pulse rate, a time production task and an evaluation of psychological effects were recorded.

6 The smoke of 250 mg of sample B provoked disruption of the time production task, increased pulse rate, and induced strong psychological reactions in four of the six subjects who received it. Similar effects, although slightly smaller, were obtained with 500 mg of sample A. On the other hand, 500 mg of sample C did not differ from placebo.

7 It is suggested that it is possible by means of animal tests to predict the potency of a marihuana sample in man.

8 In parallel experiments, Δ^9-THC was administered to other human subjects and to laboratory animals in a manner similar to that in which the marihuana samples were administered.

9 Comparison of the results between the marihuanas and Δ^9-THC showed that in man and in the laboratory animals marihuanas A and B induced effects two to four times greater than expected from their Δ^9-THC content.

10 It is suggested that there may be potentiation of the effects of Δ^9-THC by other substances present in these marihuana samples.

Introduction

The first report dealing with the administration of marihuana to humans in a double-blind procedure did not appear until 1968 (Weil, Zinberg & Nelsen, 1968). These authors and Manno, Kiplinger, Haine, Bennett & Forney (1970) have pointed out that the way the subjects smoke a marihuana cigarette, has often not been taken into consideration, and yet this may be important in determining the amount of active principles delivered to a subject. Furthermore, in many of the studies the content of (−)-Δ^9-*trans*-tetrahydrocannabinol (Δ^9-THC) in the plant material used was either not mentioned or reported imprecisely (Crancer, Diller, Delay, Wallace & Haykin, 1969; Caldwell, Myers, Domino & Merrian, 1969a & b; Manno, Kiplinger & Scholz, 1971). The chemical composition of marihuana may be another important factor. It is not yet certain whether the potencies of different marihuana samples are due only to their content of Δ^9-THC or to differences in their chemical compositions. It has been suggested that other cannabinoid compounds may interfere with

the action of Δ^9-THC (Carlini, Santos, Claussen, Bieniek & Korte, 1970; Isbell, 1971; Kubena & Barry, 1972). On the other hand, as far as we know, no previous report has been published in which a marihuana sample smoked by human beings had been previously studied in laboratory animals in order to assess its pharmacological potency. It has been suggested that certain tests in laboratory animals can detect differences in potency between samples of marihuana which correlate with their content of Δ^9-THC (Carlini *et al.*, 1970).

Recently, Renault, Schuster, Heinrich & Freedman (1971) developed a simple method of administering marihuana smoke to humans which allows for the standardization of smoke administration. Briefly, the method consists of burning a known amount of marihuana in a pipe connected to a spirometer. The subject then inhales the smoke from the spirometer under standardized conditions. In the present experiments three different samples of marihuana and Δ^9-THC were given to human beings and to laboratory animals in order to observe: a) whether the potencies of the samples were the same among the species studied; and b) whether the effects could be explained solely by the Δ^9-THC content of the samples.

Methods

Plant material

Samples A and B were apprehended by the São Paulo State Police; sample C originated from a plant cultivated at the premises of Escola Paulista de Medicina. We are grateful to Drs Orlando Rosante and Diniz Junqueira from the São Paulo Police for supplying samples A and B and to Prof. J. Ribeiro do Valle for sample C.

Gas chromatography analysis of samples A and B was kindly performed by the Research Triangle Institute of North Carolina, USA; sample C was assayed by Dr U. Claussen of Bonn University. The results are shown in Table 1.

Animal studies

Preparation of cannabis resins. Fifty g portions of the marihuanas were extracted for 12 h with petroleum ether as described by Carlini & Kramer (1965); after the petroleum ether evaporation they yielded, respectively, 12.06, 14.56 and 4.26% of resin. Suspensions of the extracted resinous material in saline (0.9% w/v NaCl solution) plus Tween-80 and a control solution were prepared as recommended by the same authors. The plant residue left after petroleum ether extraction was thoroughly dried and used thereafter as placebo in the human experiments.

Measure of corneal areflexia in rabbits (Gayer test). This was performed as described by Santos, Sampaio, Fernandes & Carlini (1966). Adult male albino rabbits weighing 2.5 to 3.5 kg were used. Suspensions of cannabis resins containing 0.5 to 4.0 mg/ml were injected at a constant speed of 0.04 ml/min through the marginal vein of the ear of adult albino rabbits. The corneal reflex was tested 10 times on each eye every 3 minutes. The abolition of at least 80% of the responses in the last three sets of tests was considered a positive assay, after which the infusion was discontinued. The total amount of cannabis resin injected was then calculated per kg of rabbit weight.

Measure of spontaneous motor activity in mice. Four photocell cages as described by Carlini & Silva (1968) were used. Male albino mice three months old and weighing 25-30 g were employed. Three or four doses in geometric progression were used for each drug, and at least 10 mice used for each dose. All drugs were given through the intraperitoneal route between 9 h 00 min and 10 h 00 min, and immediately after injections the animals were put into the cages where they remained for a period of 2 hours. The total number of light beam interruptions was counted and the values found for the control animals were taken as 100% for calculations of ED_{50} according to the log-probit-method of Miller

Table 1 Gas chromatography analysis and % of resin of marihuanas A, B and C.

Marihuana plant	*% constituents (w/w) in the plant material*				
	Δ^9-THC	*Δ^8-THC*	*Cannabinol*	*Cannabidiol*	*% extracted resin*
A	0.82	0.1	1.56	0.06	12.06
B	2.02	0.1	2.12	0.16	14.56
C	0.52	–	–	–	4.26

THC = tetrahydrocannabinol.

& Tainter (1944). Analysis of variance was performed among the control and drug treatments. When a significant difference was obtained, further comparison between control and each drug group was made by Student's *t* test. The same statistical procedure was followed in the catatonia and rope climbing experiments (see below).

Measure of catatonia in mice. The procedure of Carlini *et al.* (1970) was followed. Male albino mice three months old and weighing 25-30 g were used. The animals had their forepaws lifted on to a horizontal glass rod which was 4.5 cm from the floor. Each animal was put in this position three times, at 20 min intervals, up to 3 h after the injections. Four doses with at least 10 mice per dose were used for each compound. The total time in seconds that the animal remained in this imposed position was considered as a measure of catatonia. The theoretical maximum of catatonia (100%) shown by an animal should, therefore, be 10,800 s (equal to 3 hours). The time of catatonia recorded from the drug-injected animals was converted to percent values to allow calculation of the ED_{50} by the method of Miller & Tainter (1944).

Rope climbing performance test in rats. This test was carried out as described by Karniol & Carlini (1973); the data are expressed in the way recommended by these authors.

Human studies

Selection of subjects. For the marihuana samples, the subjects were 11 male physicians and 22 male medical students who volunteered. They had never smoked marihuana before. Eight of the subjects served in more than one experiment, and in these cases the interval between the two sessions varied from 7 to 18 days. Their ages and weights varied from 18 to 42 years and 51 to 80 kg. They were distributed into several groups (placebo or marihuana samples), balanced for age, weight and smoking habits. They were told that they were participating in a marihuana experiment, and that they might receive a small or large dose or even placebo. The experimenter administering the marihuana did not know what drug he was giving. A preliminary experiment with four other volunteers, whose results were not entered in the general data, was performed without double-blind conditions in order to give the experimenters an opportunity to choose the dosage levels of the samples. In another series of parallel experiments 14 other volunteers were exposed to three doses of Δ^9-THC as explained above (Karniol & Carlini, 1973).

Administration of the marihuana smoke to human subjects. A quantity (62.5 to 500 mg) of finely chopped plant, either A, B or C, was burned in a pipe connected through latex tubing to a 6 litre Palmer spirometer. The smoke inside the apparatus was then mixed with air to make 6 litres. Immediately after this operation (which took 2 to 3.5 min depending on the amount burned), the subjects started to inhale the mixture by mouth, with their noses blocked, according to the following schedule: 5 s of inhalation, 15 s of breath holding, 35 s of normal breathing of room air, until all 6 litres were consumed. This took six to nine cycles as described above or 5.5 to 8.5 minutes. To administer Δ^9-THC, 0.1 to 0.4 ml of an alcoholic solution containing 50 mg/ml of Δ^9-THC was added to 500 mg of placebo powder and the mixture was thoroughly mixed to allow most of the alcohol to evaporate.

Test procedure. Table 2 summarizes the procedure. Each volunteer was physically examined, and interviewed by a psychiatrist (I.G.K.) to ensure that he had no history of severe psychopathology and that he was in apparent good mental health at the time of the experiment. They were then told in detail what was to be done, how they should inhale the smoke and that they would be asked to describe their feelings. The subjects were then asked to be seated in an isolated laboratory, and to relax while pulse rate was measured 10 times at 1 min intervals. After this the subjects performed a time production task, i.e. they were asked to estimate a 60 s interval, 10 times. In the first five estimations (Estimation T1) there was no feedback from the experimenter. The following five estimations (Estimation T2) were performed with feedback, the experimenter saying either 'correct', 'too short', or 'too long' immediately after each estimation. The marihuana

Table 2 Protocol for marihuana investigation in man.

Time (min)	*Events*
0-30	Physical examination; psychiatric interview.
30-35	Explanation of the experiment.
35-55	Resting period; control pulse rate measurements.
55-65	Time production tasks T1 and T2.
65-69	Burning marihuana; preparing the spirometer.
69-76	Inhalation period.
76-96	Pulse rate measurements; time production tasks T3 and T4.
96-276	Psychiatric observation and occasional pulse rate measurements.

was then burned, the smoke collected in the spirometer, and the subjects inhaled it as described above. After inhalation, pulse rate and estimations T3 and T4 (with and without feedback) were again determined as before, but now the measurements were made concomitantly. T3 and T4 took about 15 to 20 min to complete. The subjects continued to be observed for an additional 1 to 3 h period, depending upon the symptomatology presented. About every 10-15 min the experimenter returned, sat behind them, asking questions about and recording their feelings and sensations, and then measuring their pulse rate. The psychological effects of drug action, were graded from 0 to 4, according to the subjective report of at least three symptoms in a grade:

Grade 0: nothing or slight anxiety.

Grade 1: slight feeling of well-being; feeling of lightness; paraesthesia in extremities; slight difficulty in concentration; dizziness; somnolence; cold hands and sweating.

Grade 2: definite feeling of well-being; euphoria; colours are brighter; intense paraesthesia; uninterested by the surroundings; some difficulty in reporting feelings; sometimes slight sensation of fear; intense difficulty in concentration.

Grade 3: marked sensation of euphoria intercalated with moments of apprehension; intense introspection with resistance to describing feelings; sensation of being watched; sounds are clearer and colours are brighter; laughing without reason; concentration almost impossible due to the rapid flow of ideas; extremities very heavy; unable to visualize intact objects with eyes closed (e.g. watch seen without numbers or hands).

Grade 4: feelings of well-being followed later by panic; intense sensation of being watched; coherent thoughts impossible due to the rapid flow of ideas; in general, subject knows what is happening, but loses the knowledge from time to time and panic starts; cinaesthesia; striking visual hallucinations.

As explained above, in another series of parallel experiments several doses of Δ^9-THC were given to rats, mice, rabbits and to human volunteers. The overall data and some aspects of the results will be given here; the details of the experimental procedure and other data are given elsewhere (Karniol & Carlini, 1973).

Results

Human studies

General physical reactions of the subjects. Thirty-one of the subjects receiving marihuanas A, B or C did not present any marked physical signs or symptoms after the inhalation of smoke. Occasionally, paroxysmal episodes of coughing or a sensation of nausea were reported. However, two of the subjects who smoked 125 mg of marihuana B and 500 mg of marihuana A respectively, complained of faintness, vague physical discomfort and profuse sweating. The blood pressure of one of these subjects fell to 60/30 mmHg from a pre-drug level of 100/80 mmHg. These symptoms were quickly relieved in both subjects by putting down their heads below body level. Such effects did not appear in the volunteers receiving Δ^9-THC.

Pulse rate. The larger doses of Δ^9-THC and of marihuanas A and B increased pulse rate. The effect was seen even at the first measurement after the end of inhalation and reached a peak after a maximum of 8 min, returning to levels near normal within 20 to 50 minutes. The increase was dose-dependent. Table 3 shows the average results expressed as a percentage of the pre-smoking period. Plants A and B significantly increased pulse rate when compared to placebo and B was nearly twice as active as plant A; plant C had practically no effect. Marihuanas A and B induced effects of greater magnitude than were expected from their Δ^9-THC content. Thus, 500 mg of A (containing 4.0 mg of Δ^9-THC) and 250 mg of B (5.0 mg of Δ^9-THC) induced an increase of pulse rate comparable to that obtained with 10 mg and larger than that seen with 5.0 mg of Δ^9-THC.

Time production task. Δ^9-THC, and marihuanas A and B affected estimation T3 significantly (Table 4). Sample C did not differ from placebo. Again, plant B was more active than plant A. The figures in Table 4 represent absolute deviations from 1 min (above or below). The direction of such deviations can be seen in Fig. 1, for the largest doses of the three marihuana samples. After marihuanas A and B, respectively, 41 and 67% of the subjects estimated less than 55 s as being 1 minute. As no dose-effect relationships were obtained in this measure, direct comparison with Δ^9-THC is difficult. Nevertheless, the effects of 250 and 500 mg of plant A (containing 2.0 and 4.0 mg of Δ^9-THC) and of 125 and 250 mg of plant B (2.5 and 5.0 mg of Δ^9-THC) were similar to those of 5 and 10 mg of Δ^9-THC. Although objectively the subjects overestimated the length of time that had elapsed, they later said they had the impression that time was passing slowly and thought they had underestimated the passing of 60 seconds. However, these effects decreased when estimation was helped by feedback (estimation T4).

Table 3 % increase in pulse rate in man after smoking varying amounts of three samples of marihuana and of Δ^9-tetrahydrocannabinol (Δ^9-THC).

Drug	*Amount of plant smoked (mg)*	*Corresponding amount of Δ^9-THC (mg)†*	*Number of subjects†*	*% difference in pulse rate (±s.e.)‡*
Placebo A or B	250 or 500	–	7	+8.3 ± 3.0
Δ^9-THC	5§	5.0	5	+19.0 ± 5.7
	10§	10.0	4	+28.6 ± 7.3*
	20§	20.0	5	+51.5 ± 4.5***
Marihuana A	125	1.0	4	+13.4 ± 2.7
	250	2.0	6	+22.9 ± 3.9*
	500	4.0	5	+33.7 ± 4.2***
Marihuana B	62.5	1.2	4	+15.0 ± 5.5
	125	2.5	4	+31.4 ± 3.9***
	250	5.0	6	+36.0 ± 7.1**
Marihuana C	250	1.2	3	+10.9 ± 2.4
	500	2.5	3	+15.5 ± 7.8

† The same for Tables 4 and 5.
‡ % was calculated taking the average of the last three measurements before smoking as 100%, and comparing it with the greatest value after smoking. Student's *t* test, comparison made with placebo group: $*P \leq 0.02$; $**P \leq 0.01$; $***P \leq 0.005$.
§ A small volume (0.1-0.4 ml) of an alcoholic solution containing 50 mg/ml Δ^9-THC was added to 500 mg placebo powder and the mixture was thoroughly mixed to allow most of the alcohol to evaporate (see Karniol & Carlini, 1973). (Same for Tables 4 and 5.)

Table 4 Effects of three samples of marihuana and of Δ^9-tetrahydrocannabinol (Δ^9-THC) on a time production task in man.

Drug used	*Amount of plant smoked (mg)*	*Seconds of deviation (±s.e.) in producing 1 min, before (T1 and T2) and after (T3 and T4) drug inhalation*			
		T1†	*T2†*	*T3*	*T4*
Placebo A and B	250 or 500			5.6 ± 1.5	6.2 ± 2.0
Δ^9-THC	5			13.3 ± 3.2***	5.9 ± 2.1
	10			9.9 ± 4.6*	6.0 ± 2.6
	20			18.0 ± 2.8***	5.8 ± 3.2
Marihuana A	125			9.4 ± 4.0**	6.1 ± 3.1
	250	12.5 ± 1.1	5.6 ± 0.4	9.5 ± 2.6***	6.2 ± 2.0
	500			8.9 ± 4.1*	7.8 ± 3.3
Marihuana B	62.5			4.8 ± 2.0	4.9 ± 1.7
	125			10.7 ± 3.1****	6.5 ± 2.3
	250			13.6 ± 4.0****	6.1 ± 2.2
Marihuana C	250			7.0 ± 3.2	5.8 ± 2.7
	500			3.9 ± 1.9	6.0 ± 1.8

† T1 and T2 represent the average of 20 estimations selected according to a table of random digits (Wyatt & Bridges, 1967) from the total of estimations performed by all subjects (33 subjects x5 estimations for each = 165 estimations).
The asterisks at column T3 indicate statistical differences from placebo group. Student's *t* test: $*P \leq 0.1$; $**P \leq 0.05$; $***P \leq 0.02$; $****P \leq 0.005$.

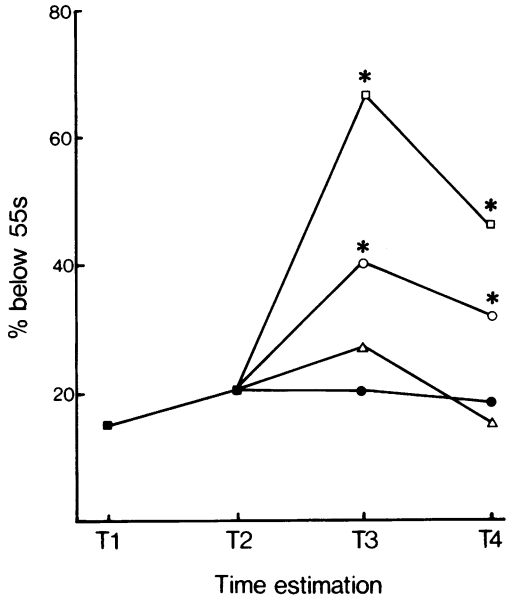

Fig. 1 Effects of three samples of *Cannabis sativa* and of placebo on a time production task of volunteers. Times T1 and T2 (■) indicate the control estimations, before drug inhalation. Note that the percentage of subjects producing 1 min as below 55 s increased significantly (T3) after smoking 500 mg of plant A (○) and 250 mg of B (□). 500 mg of marihuana C (△) and placebo (●) were inactive. The asterisks indicate statistically significant differences from placebo group (Chi square test; $P \leq 0.05$).

Psychological effects. Table 5 shows the psychological reactions reported by the subjects. The larger the doses of Δ^9-THC and of plants A and B, the larger the number of subjects showing positive reactions. For example, with 250 mg of B, four of the six volunteers gave reactions graded three and four. On the other hand, two of the seven subjects who smoked placebo reported discrete changes that were recorded as grade one; subjects receiving marihuana C behaved as the placebo group. Again the effects of plants A and B were larger than expected from their Δ^9-THC contents. For example, 250 mg of B (containing 5.0 mg of Δ^9-THC) induced more reactions graded three and four than 10 or 20 mg of Δ^9-THC. It was further observed that the psychological effects started around 10 min after the end of inhalation and reached a maximum 20 to 30 min later, subsiding within 1 to 3 hours. The peak of psychological disturbances, therefore, did not coincide in time with the peak of pulse rate effect. In general, the subjects who showed the largest psychological disturbances (grades three and four) were also those who showed the largest pulse rate and time production changes.

Animal studies

Corneal areflexia in rabbits. Table 6 shows the results of these tests. Resin B was significantly more active than resins A and C but did not differ significantly from Δ^9-THC. The actual net content of Δ^9-THC in the injected amounts of resins, as

Table 5 Distribution of subjects in a scale of psychological reactions, according to the effects produced by samples of marihuana and by Δ^9-tetrahydrocannabinol (Δ^9-THC).

Amount of plant smoked (mg)	*Number of subjects with psychological reactions graded as:* 0	1	2	3	4
Placebo 250 or 500 mg	5	2	0	0	0
Δ^9-THC 5 mg	1	4	0	0	0
10 mg	1	0	2	0	1
20 mg	0	1	2	2	0
Marihuana A 125 mg	2	1	1	0	0
250 mg	1	3	1	1	0
500 mg	0	2	2	1	0
Marihuana B 62.5 mg	3	1	0	0	0
125 mg	1	2	1	0	0
250 mg	0	1	1	3	1
Marihuana C 250 mg	2	1	0	0	0
500 mg	2	1	0	0	0

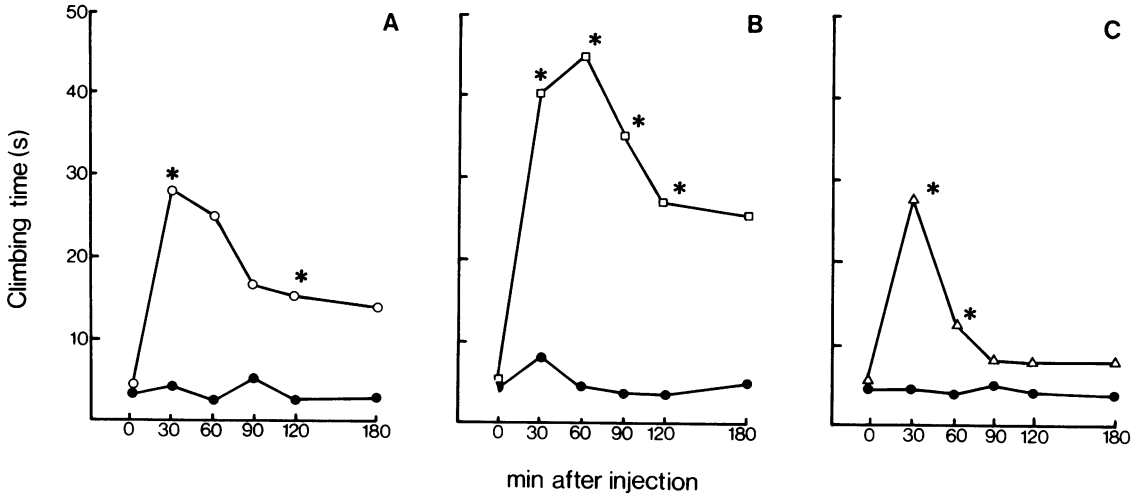

Fig. 2 Effects of marihuana resins A, B and C on the rope climbing performance of trained rats. (●) Climbing time in seconds after injection of 1.0 ml/kg of control solution; (○, □, △) climbing time in seconds after the injection of 20 mg/kg of, extracts A, B and C respectively. The results are for an average of five animals per drug. The asterisks indicate significant differences from controls (Student's t test; $P \leq 0.05$).

calculated from the chemical data of Table 1, are shown in the last column of Table 6. On the other hand, by considering the values obtained in this test with Δ^9-THC, the yield of resins obtained directly from the plants and their values for the Gayer test, it was calculated (Carlini, Santos, Claussen, Bieniek & Korte, 1970) that marihuanas A, B and C should contain approximately 4.8, 9.3 and 1.3% Δ^9-THC, respectively, to explain their activities in the Gayer test. In other words, marihuana resins induced corneal areflexia with doses smaller than expected from their Δ^9-THC content.

Spontaneous motor activity in mice. Table 7 shows the decrease in motor activity produced by 10-80 mg/kg of Δ^9-THC, resins A, B and C, and the ED_{50}'s calculated from these data. The estimated ED_{50} for resin A (82.5 mg/kg) was extrapolated from the curve, as it was outside the experimental dose range. The same occasional need to extrapolate in order to obtain estimates of ED_{50}'s is also seen in Tables 8 and 9. Resin B was more active than A and C in reducing the motor activity of mice. On the other hand, the resins were less active than Δ^9-THC. However, as seen in the last column of Table 7, resins A and B were more active than would be expected from their Δ^9-THC contents.

Catatonia activity in mice. The dose-response curves for Δ^9-THC and sample B were apparently not parallel, which made a potency comparison difficult (Table 8). However, a new experiment

Table 6 Effects of resins obtained from three samples of marihuana, and of Δ^9-tetrahydrocannabinol (Δ^9-THC) on the corneal reflex of rabbits (Gayer test).

Cannabis preparation	*Number of animals*	*mg/kg (±s.e.) Δ^9-THC or of resin to abolish corneal reflex*	*Corresponding amount of Δ^9-THC (mg/kg)*
Δ^9-THC	5	0.101 ± 0.01	0.101
Resin A	6	0.250 ± 0.02* †	0.017
Resin B	7	0.159 ± 0.02	0.021
Resin C	8	0.320 ± 0.03** †	0.033

Student's t test, comparison made with Δ^9-THC: *$P \leq 0.05$; **$P \leq 0.01$; comparison made with resin B: †$P \leq 0.05$.

Table 7 Activity of resins obtained from three samples of marihuana, and of Δ^9-tetrahydrocannabinol (Δ^9-THC), on the spontaneous motor activity of mice.

Cannabis preparation	*Motor activity (number of light beam interruptions ± s.e.) within 2 h of injections of Δ^9-THC or resin:*					*ED_{50} in mg/kg (±s.e.) to reduce motor activity*	*Corresponding amount of Δ^9-THC (mg/kg)*
	0 mg/kg	*10 mg/kg*	*20 mg/kg*	*40 mg/kg*	*80 mg/kg*		
Control	523 ± 89	–	–	–	–	–	–
Δ^9THC	–	261 ± 26	244 ± 48**	184 ± 31***	174 ± 25***	18.7 ± 7.0	18.7
Resin A	–	540 ± 94	356 ± 50	348 ± 75	233 ± 55*	85.2 ± 79.5	5.7
Resin B	–	433 ± 78	281 ± 44*	206 ± 38**	194 ± 30**	37.3 ± 26.8	5.1
Resin C	–	328 ± 56	544 ± 102	363 ± 51	–	–	–

Student's *t* test, comparison made with control solution: $*P \leqslant 0.05$; $**P \leqslant 0.005$; $***P \leqslant 0.001$.

Table 8 Catatonia-inducing properties in mice of resins obtained from three samples of marihuana and of Δ^9-tetrahydrocannabinol (Δ^9 -THC).

Cannabis preparation	*Catatonia (in s ± s.e.) within 3 h of injection of Δ^9-THC or resin:*						*ED_{50} in mg/kg (±s.e.) to induce catatonia*	*Corresponding amount of Δ^9-THC* (mg/kg)
	0 mg/kg	*2.5 mg/kg*	*5.0 mg/kg*	*10 mg/kg*	*20 mg/kg*	*40 mg/kg*		
Control	103 ± 33	–	–	–	–	–	–	–
Δ^9-THC	–	1063 ± 259*	1832 ± 329*	2854 ± 610**	–	–	34.1 ± 10.4	34.1
Resin A	–	–	–	1200 ± 484*	3208 ± 733**	3521 ± 698**	52.5 ± 36.1	3.5
Resin B	–	–	–	472 ± 123**	3454 ± 987*	5717 ± 621***	33.9 ± 10.0	4.7
Resin C	–	–	–	181 ± 95	1425 ± 629	1740 ± 834	89.3 ± 33.5	10.8

Student's *t* test, when compared to the control group; $*P \leqslant 0.05$; $**P \leqslant 0.005$; when compared with same dose of resin A: $***P \leqslant 0.05$.

Table 9 Activity of three cannabis resins and of Δ^9-tetrahydrocannabinol (Δ^9 -THC) on the rope climbing performance of rats.

Cannabis preparation	*Rope climbing delay expressed in cm^2 (±s.e.) after the injection of Δ^9-THC or resin:*					*ED_{50} in mg/kg (±s.e.) to reduce climbing time*	*Corresponding amount of Δ^9-THC (mg/kg)*
	0 mg/kg	*5 mg/kg*	*10 mg/kg*	*20 mg/kg*	*40 mg/kg*		
Control	15.4 ± 5.5	–	–	–	–	–	–
Δ^9-THC	–	16.5 ± 2.0	190.5 ± 62.7*	330.0 ± 11.8***	–	11.3 ± 3.8	11.3
Resin A	–	–	55.1 ± 15.1	107.8 ± 39.0	213.6 ± 46.5**	26.3 ± 13.8	1.7
Resin B	–	16.5 ± 4.1	57.0 ± 15.6*	227.2 ± 35.4***	–	13.5 ± 5.4	1.8
Resin C	–	–	18.9 ± 5.5	50.3 ± 8.8**	103.3 ± 9.5***	50.2 ± 23.5	6.1

Student's *t* test, comparison made with the control group: $*P \leqslant 0.05$; $**P \leqslant 0.025$; $***P \leqslant 0.005$.

carried out with Δ^9-THC gave an ED_{50} of 37.1 ± 12.0 mg/kg. Furthermore, this lack of parallelism may be insignificant compared to the large limits of error found in the catatonia test. Thus, it seems that resin B, in this test, was also more active than resins A and C. As seen in Table 8, their respective ED_{50}'s were 33.9, 52.5 and 89.3 mg/kg. On the other hand, sample B was as active as Δ^9-THC. However, the actual contents of Δ^9-THC in the resins were smaller than expected, as can be seen in the last column of Table 8.

Rope climbing performance of rats. Figure 2 and Table 9 summarize the results. Again, resin B was the most active and C the least active. No difference was found between resin B and Δ^9-THC. Again, as shown in the last column of Table 9, the Δ^9-THC contents of the resins were smaller than expected.

Discussion

Δ^9-THC and two of three samples of marihuana smoked by volunteers under a standardized procedure, increased pulse rate, disrupted a time production task and induced psychological reactions which varied from mild to marked distortions in mood and perception. These effects, although they did not occur concomitantly, varied quantitatively according to the plant sample used. As a rule, the dose and/or the sample that was more active in changing pulse rate was also more efficient in disrupting the time production task and in inducing psychological symptoms. The peak pulse rate changes preceded peak psychological symptoms by 10 to 20 minutes. This symptomatology has been described before for marihuana and Δ^9-THC (Weil *et al.*, 1968; Manno *et al.*, 1970; Manno *et al.*, 1971; Isbell & Jasinski, 1969; Tinklenberg, Melges, Hollister & Gillespie, 1970; Hollister, 1970; Melges, Tinklenberg, Hollister & Gillespie, 1970; Isbell, Gorodetzsky, Jasinski, Claussen & Korte, 1967).

The effects on the time production task deserve further comment. The disruption was clearly observed at estimation T3, but decreased at estimation T4 when subjects were given feedback. This could be explained on the supposition that when the drugged subjects were left undisturbed, without feedback, the drug effects could manifest themselves more freely; and, as a consequence, the rapid flow of ideas could more readily impair concentration. Another interpretation could be that the drug's effect on the time task wears off very rapidly so that at estimation T4 the subjects were again able to estimate correctly. However, we favour the former hypothesis. It might be possible that the experimenter can limit the psychological effects of marihuana, and, when drugged subjects are given batteries of tests and other procedures, the total manifestations of a marihuana 'high' may not be seen. In this respect, it is interesting that Tinklenberg *et al.* (1970) reported that when the subjects were exhorted to try as hard as possible they could mitigate considerably the influence of marihuana on attention.

Comparison of potencies was made between Δ^9-THC and the marihuana samples, or among the samples, in spite of the large limits of error found in some experiments (for example, see Tables 8 and 9). However, as these limits of error are usually found in experiments with cannabis and as in all the experiments performed with rabbits, mice, rats and man, the same relative potencies were obtained, the differences found were taken into consideration.

Thus, the activity of marihuana B was approximately double that of A when pulse rate, time production task or psychological reactions were measured. Marihuana C, on the other hand, was practically inactive. The results obtained from rabbits, mice and rats paralleled those obtained in man, showing that marihuana B was twice as active as marihuana A, and that C possessed weak activity. This is evidence that marihuana has consistent effects depending upon potency amongst these four species and suggests that it is possible through animal tests to predict the potency of a marihuana sample in man. Thus, 250 to 500 mg of a plant, the petroleum ether extract of which abolishes the corneal reflex of rabbits at approximately 0.150 mg/kg, prolongs rope climbing time of rats at an ED_{50} of about 13 mg/kg, reduces motor activity and induces catatonia in mice at ED_{50}'s of 37 and 33 mg/kg respectively, can be expected under the conditions we have stated to induce strong psychological reactions in human subjects.

According to the chemical analysis (Table 1), 250 mg of sample B and 500 mg of samples A and C should contain respectively 5.0, 4.0 and 2.5 mg of Δ^9-THC. However, the results we have obtained with the human volunteers indicate that the activity of 250 mg of sample B and 500 mg of sample A was comparable to the effects of at least 10 mg of Δ^9-THC. The same discrepancy was also obtained with the laboratory animals; thus, as mentioned before in the **Results**, according to the Gayer test, 4.8 and 9.3% respectively of Δ^9-THC would be expected in samples A and B. Results greater than expected were also obtained in rats and mice.

These data suggest that the potency of the marihuana samples we have used cannot be

explained solely on the basis of their content of Δ^9-THC. They can not be explained either by the amount of Δ^8-THC in samples A and B which was 0.1%. (We are grateful to National Institute of Mental Health for performing these assays.) It is possible, however, that a synergistic action of other compounds with Δ^9-THC occurred. For example, Kubena & Barry (1972) suggested such a role for the *n*-propyl analogue of Δ^9-THC. It is interesting in this respect that not only the *n*-propyl but also a methyl analogue of Δ^9-THC has been identified in our sample B (Vree, Breimer, Van Ginneken & Van Rossum, 1972). On the other hand, as we have suggested before (Karniol & Carlini, 1972), the relatively large amount of cannabinol (Table 1) present in our samples A and B could be potentiating the effects of Δ^9-THC.

It is also pertinent that interaction between Δ^9-THC and other cannabis constituents may not be limited to potentiation. For example, cannabidiol has recently been found to inhibit some effects of Δ^9-THC in laboratory animals (Karniol & Carlini, 1973b). This fact does not invalidate the present results, because, as shown in Table 1, marihuanas A and B are practically devoid of cannabidiol.

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