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The ring test: a quantitative method for assessing the 'cataleptic' effect of cannabis in mice

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Summary

1. A bioassay for cannabis, called the ring test, has been developed in which the percentage of the total time spent on a horizontal wire ring during which a mouse remains completely immobile is recorded.

2. The effect of cannabis on mobility is a dose-related, graded response.

3. Threshold doses of cannabis extract are 12·5 mg/kg when injected intravenously, and 100 mg/kg when injected intraperitoneally or subcutaneously.

4. The method provides a measure of the 'cataleptic' effect of cannabis. Chlorpromazine in doses of 1 mg/kg upwards also produces the effect but barbitone does not.

5. It is concluded that Δ^1-tetrahydrocannabinol (Δ^1-THC) is largely responsible for the effect of cannabis extract on mobility; the potency ratio of Δ^1-THC to cannabis extract is between 10 and 20. Δ^1-Tetrahydrocannabidivarol (Δ^1-THD) also affects mobility but is less active than Δ^1-THC. Cannabidiol has no effect when injected intraperitoneally in doses up to 100 mg/kg.

Introduction

A new quantitative method has been developed for assaying cannabis biologically and is described here. The method exploits an effect first observed by Loewe (1946) who reported that after an adequate dose of parahexyl or 'charas' tetrahydrocannabinol, a mouse placed in a prone position and supported only at its thighs and jaws maintained this extended position until an adequate stimulus aroused it. A preliminary account of some of the experiments has been published (Gill, Paton & Pertwee, 1970). The method (the 'ring test') was demonstrated in September 1971 at a meeting of the British Pharmacological Society.

Methods

The apparatus consists of a horizontal wire ring of 5·5 cm diameter which is attached at a point on its circumference to the top of a 16 cm length of 12 SWG gauge stainless steel tubing held vertically. The wire ring is constructed of 16 SWG gauge tinned copper wire.

An untreated mouse placed on the wire ring remains almost continuously active. It explores its new environment, sometimes escaping from the ring by jumping or falling off, or, more usually by climbing down the vertical tubing supporting the ring.

In contrast, the exploratory activity of a mouse treated with an adequate dose of cannabis is repeatedly interrupted by periods in which all voluntary movements

of tail, limbs, trunk, snout and whiskers cease. Only respiratory movements remain. During these periods of immobility, the mouse adopts one of several positions, a common one being to lie across the wire ring, its belly gradually sagging so that the animal's position becomes progressively less stable. Return to normal activity is usually a transient event which occurs sometimes only when the animal has fallen from the ring, although more usually when it seems to be in imminent danger of so doing. A transient return to normal activity also occurs if the mice are touched. The proportion of the total time spent on the ring during which an animal treated with cannabis remains motionless is dose-related and this forms the basis of the assay method.

The assay is performed by lifting up the mouse by its tail and placing the animal gently across the wire ring. During the following 5 minutes the sum of the times during which the mouse remains motionless is noted to the nearest second. The criterion for immobility is the absence of all voluntary body movements except for those associated with breathing. The characteristic passive sagging movements already described are also excluded. Lack of snout and whisker movements provides an easily recognizable endpoint for the onset of immobility and serves to make the test reasonably objective. Immobility is expressed as an 'immobility index', defined as that percentage of the total time spent on the ring during which the animal remains motionless.

Preferably, the procedure for performing the ring test should be identical for each of the mice subjected to the test. However, escapes defined operationally as jumps or falls from the ring or as excursions to the foot of the tubing supporting the ring often occur before the end of a test period. In such cases, either the test could be continued by replacing the mouse on the ring, so subjecting it to additional handling, or it could be terminated at the moment of escape, so reducing the time period spent on the ring. The standard procedure that has been adopted is as follows: if a mouse has already spent at least half the usual test period (i.e. 2·5 min) on the ring before an escape is made, the test is terminated and the immobility index is calculated for the shortened test period. It has been found that indices calculated from a 2·5 min test period are only slightly less than indices calculated from the full 5 min period. For instance, with a 5 min test period, the mean index of a group of 6 mice treated with cannabis extract (500 mg/kg i.p.) was 62 ± 5 whereas the index calculated from data obtained during the first 2·5 min of the test period was 55 ± 6; there was no significant difference ($P > 0{\cdot}05$) between the two values.

The procedure adopted when a mouse leaves the ring within 2·5 min is to return the animal to the ring for a further 3 minutes. In this case the index is calculated for time spent on the ring both before and after the escape. If mice escape repeatedly the test is terminated after 5 successive escapes and in any event is concluded within 6 minutes. (Most mice which have escaped 5 times continue to escape repeatedly if placed back on the ring.) To reduce the incidence of escapes from the ring, mice are always placed on the ring facing away from the point at which it joins the vertical supporting rod.

Drugs were administered to mice intraperitoneally, subcutaneously or intravenously. The injection volume was usually 0·1 ml/25 g. Each intravenous injection was made through a cannula, inserted into a lateral tail vein. The mice used were non-fasted, adult, males weighing 20–30 g and supplied by A. J. Tuck (Tuck

No. 1 strain). Cannabis was obtained as an ethanolic tincture (BPC 1949), prepared commercially by extracting with cold ethanol, leaves and flowers of *Cannabis sativa* of Pakistani origin, evaporating the solvent and dissolving the resinous residue in free ethanol. The residue has been found (Paton & Pertwee, 1972) to contain 6·4% by weight of Δ^1-tetrahydrocannabinol (Δ^1-THC), 3·4% by weight of Δ^1-tetrahydrocannabidivarol, the *n*-propyl analogue of Δ^1-THC (Δ^1-THD: Gill, 1971) and 3·6% by weight of cannabidiol. The crude cannabis resin, the Δ^1-THC and the Δ^1-THD were each prepared for administration by dispersion in a mixture of Tween 80 and a 0·9% NaCl solution. The resin was prepared by removing solvent *in vacuo* from the cannabis tincture. We are indebted to Dr. E. W. Gill for Δ^1-THC and Δ^1-THD extracted from the petrol-ether soluble fraction of crude resin. The solutions of crude cannabis contained 2·5 parts of drug to 1 part of Tween 80 by weight. Solutions of Δ^1-THC and cannabidiol contained 2 parts of Tween 80 to 1 part of material by weight, whereas the ratio of Tween 80 to material in solutions of Δ^1-THD was 12. Control injections were made with solutions containing appropriate amounts of Tween 80 in a 0·9% NaCl solution.

Chlorpromazine and barbitone were each dissolved in a 0·9% NaCl solution and injected subcutaneously. They were used respectively as the hydrochloride and the sodium salt and doses are quoted in terms of the salts.

Finally, differences between drug and control groups were evaluated by Student's *t* test and limits of error are expressed as standard error of the means. Where Tween was used as a vehicle, control injections (apart from one exception, see Table 1) contained doses of Tween equal to or greater than those used in the appropriate drug injections. It is noteworthy that over the range of Tween doses used, no relationship between immobility index and dose level of Tween could be detected.

Results

Experiments with cannabis extract

Cannabis injected intraperitoneally or intravenously can induce hypothermia in mice at room temperature (Gill *et al.*, 1970) and because hypothermia might itself depress spontaneous activity and hence increase the immobility index, the first experiments with the ring apparatus were carried out at 30–32° C, a range of environmental temperature at which cannabis has no effect on the body temperature of mice (Paton & Pertwee, 1972). Table 1 shows the results obtained. It was found that increases in the immobility index could be produced by cannabis extract in the absence of hypothermia and that both the level and the duration of the effect were dose-related.

The drug was administered either intraperitoneally or subcutaneously. By both routes, doses of cannabis extract ranging upwards from 100 mg/kg increased the immobility index significantly ($P<0·05$) above control values, the effect appearing within 30 or 60 min of injection. The maximal effects produced by subcutaneous and intraperitoneal dosage did not differ significantly, and the onset of the peak effect of the drug, defined as the earliest time after injection at which the mean immobility index of a group of mice is no longer significantly different from the maximum value observed, occurred within 60 min of injection by either route.

To test whether hypothermia influences the effect of cannabis extract on the immobility index, experiments were also performed at room temperature (Table 2): 60 min after intraperitoneal injections of 200 or 500 mg/kg (doses of cannabis, effective in inducing hypothermia at room temperature), the immobility indices did not differ significantly ($P>0{\cdot}1$) from the corresponding values obtained when the experiments had been performed at 30–32° C.

TABLE 1. *Effect of cannabis extract on the mean immobility index* (±S.E.M.) *of groups of mice at 30–32° C measured at several intervals after injection of the drug (i.p. or s.c.)*

Experiment	Dose (mg/kg)		Mean immobility index (±S.E.M.) at 30, 60, 120 and 240 min after injection			
	Cannabis	Tween	30	60	120	240
				Intraperitoneal route		
a	100	40	27± 7 (6)	36±4 (6)*	40±6 (6)	38± 7 (6)
	—	60	13± 3 (6)	20±5 (6)	35±5 (6)	39± 7 (6)
b	200	80	44± 3 (36)*	48±4 (18)*	39±8 (6)*	28± 3 (6)
	—	100	12± 5 (6)	9±3 (6)	20±3 (6)	34± 3 (6)
c	500	200	54± 5 (12)*	66±5 (12)*	67±6 (6)*	56± 7 (6)*
	—	200	5± 2 (6)	16±2 (6)	25±5 (6)	37± 5 (6)
d	1,000	400	51±10 (6)*	70±3 (6)*	72±3 (6)*	70± 3 (6)*
	—	400	17± 5 (6)	34±6 (6)	38±9 (6)	37± 4 (6)
e	1,500	600	64± 3 (15)*	—	—	—
	—	800	19± 5 (9)	—	—	—
				Subcutaneous route		
f	100	40	24± 4 (14)*	30±7 (8)	37±6 (8)	39± 6 (8)
	200	80	27± 4 (6)*	43±6 (6)	55±5 (6)*	55±11 (6)
	—	60	14± 3 (6)	29±8 (6)	25±5 (6)	34± 7 (6)
g	500	200	25± 7 (6)	75±5 (6)*	78±3 (6)*	71± 6 (6)*
	—	200	23± 5 (6)	25±5 (6)	26±6 (6)	37± 7 (6)
h	1,000	400	35± 8 (6)	59±6 (6)*	59±6 (6)*	63± 9 (6)
	—	400	13± 6 (6)	22±7 (6)	27±6 (6)	37± 8 (6)

Differences at each time after injection between drug and Tween treatments were evaluated by Student's *t* test. In each experiment (apart from one exception in experiment f), the comparisons were made between one or more drug treatments and a Tween treatment in which the dose of Tween administered was equal to or greater than that used in the drug treatments. Significant differences ($P<0{\cdot}05$) are denoted by asterisks. The digits in parentheses denote the number of mice used. Mice were usually tested 4 times but in experiments b, c, and f, some mice were tested only 30 min or 30 and 60 min after injection.

TABLE 2. *Effect of cannabis extract on the mean immobility index* (±S.E.M.) *of groups of mice at 20° C measured at several intervals after injection of the drug (i.p.)*

Dose mg/kg		Mean immobility index (±S.E.M.) at 60, 240, or 420 min after injection		
Cannabis extract	Tween	60	240	420
200	80	37±6 (6)*	19±4 (6)	20±8 (6)
500	200	65±5 (6)*	61±6 (6)*	53±6 (6)*
—	200	10±4 (6)	9±3 (6)	18±5 (6)

Differences at each time after injection between drug and Tween treatments were evaluated by Student's *t* test. The comparisons were made between the drug treatments and a Tween treatment in which the dose of Tween administered was equal to or greater than that used in the drug treatment. Significant differences ($P<0{\cdot}05$) are denoted by asterisks. The digits in parentheses denote the number of mice used. All mice were tested 3 times and the experiments were performed at room temperature (20° C).

Intravenous injections of cannabis were effective in raising the immobility indices of mice at room temperature at doses which ranged from 12·5 mg/kg upwards (Fig. 1); a dose of 6·25 mg/kg was ineffective. The increases induced by doses of 12·5 mg/kg and 50 mg/kg appeared within 5 min of injection and at this time were not significantly different from the maximum effects. Both the magnitude of the peak effects and the duration of the effect were dose-related.

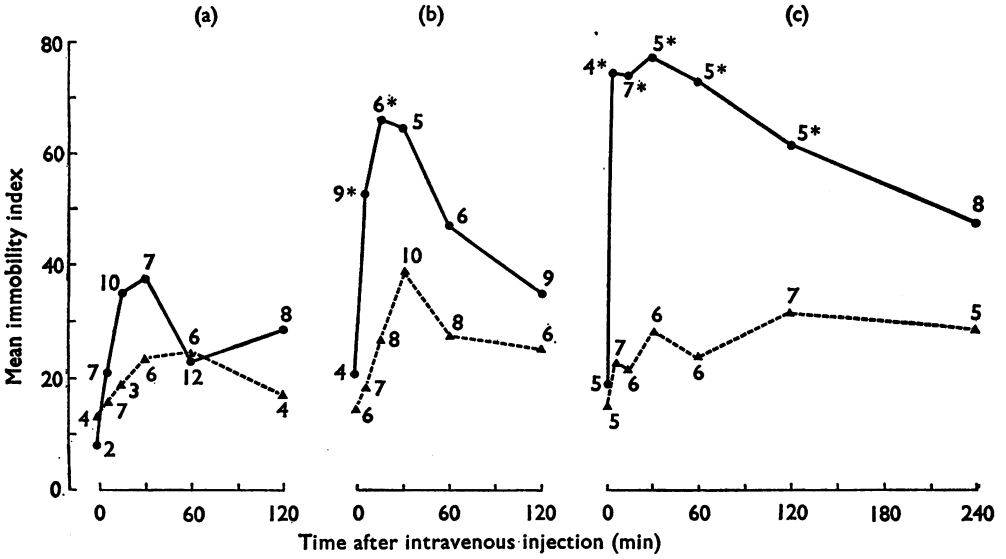

FIG. 1. Effect of cannabis extract on the immobility index of mice at 20° C measured sequentially once before (at zero time) and at several intervals after treatment with (a) 6·25 mg/kg (5 mice), (b) 12·5 mg/kg (5 mice), or (c) 50 mg/kg (6 mice), of the drug (●——●) or with control injections of Tween 80 (▲- - - -▲). Asterisks denote significant increases in the immobility index ($P<0·05$) and the digits adjacent to each point denote the standard error of the means (S.E.M.).

Escapes from the ring frequently occurred in all these experiments and as a result, test periods varied and were often shorter than 5 minutes. Thus data collected in experiments described above, 30 min after intraperitoneal injections of cannabis (200, 500, 1,000 and 1,500 mg/kg) or Tween (100, 200, 400 and 800 mg/kg) showed that the mean test periods following these two treatments were respectively 264 ± 17 s and 228 ± 10 seconds. It was also found that the proportion of mice to escape was greater in the Tween experiments (70%) than in the cannabis experiments (48%). However, no relationship was detected between the incidence of escapes and the dose levels either of Tween or of cannabis.

The effect of repeated testing

Repeated placing of untreated mice on the ring is accompanied by a progressive increase in the immobility index; this effect is illustrated in Figure 2. It was necessary, therefore, to assess how far such repetitions of the test, for instance in following the time course of cannabis action, would distort the assay.

First, it was found that a single repetition of the test performed on untreated mice (Fig. 2) or on mice injected with Tween or with cannabis did not significantly ($P>0·1$) alter the index. Thus there was no significant difference between the

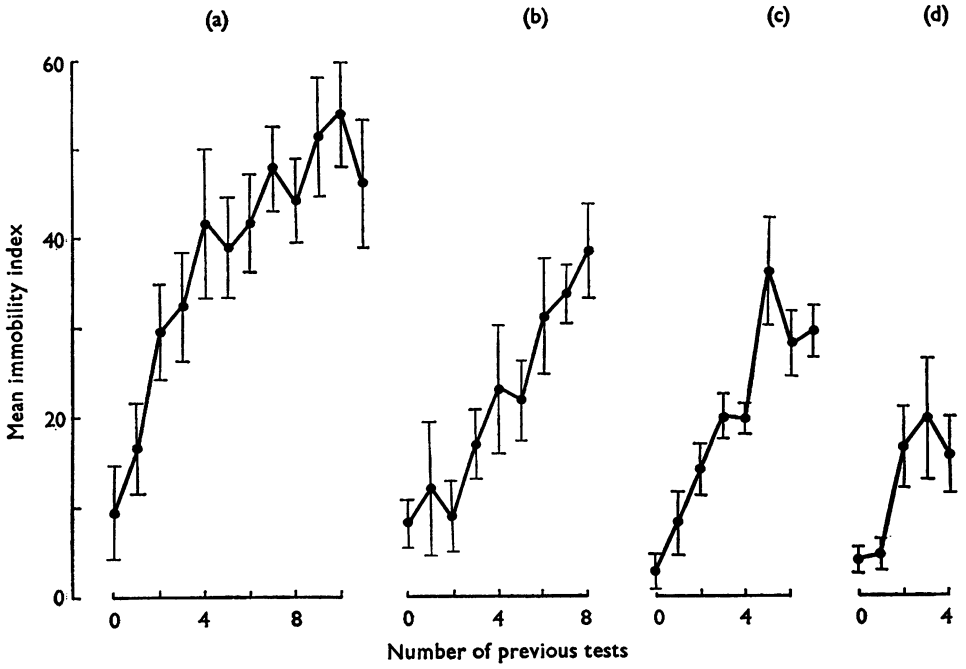

FIG. 2. Effect of performing the ring test (a) every 15 min (b) every 30 min (c) every hour (d) every 2 h, on the mean immobility index (±S.E.M.) of groups of 6 untreated mice.

mean immobility indices of two groups of 6 mice tested 60 min after subcutaneous injections of Tween 80 (60 mg/kg at 30–32° C) one group having been tested once only (18 ± 7) and the second group having also been tested 30 min after injection (29 ± 8). Identical patterns of testing performed on two groups of 6 mice pre-treated intraperitoneally at 30–32° C with 500 mg/kg of cannabis extract produced similar results. The group of mice tested only 60 min after treatment with cannabis had a mean immobility index of 66 ± 6; the second group, tested both 30 and 60 min after injection, had a mean index of 57 ± 3 at the latter time.

Second, it was found that the performance of more than two ring tests on the same animals could produce significant increases ($P<0{\cdot}05$) in the immobility index. It was found (see Fig. 2) that when the ring test was repeated at intervals of 15, 60 or 120 min, significant increases in the index occurred in the third test. Repetition at hourly intervals produced significant increases in the sixth test. It is of interest that this adaptation to the test procedure appears to be related more to the number of tests performed than to the interval between tests.

The sizes of the peak effects of cannabis were determined (see above) from data obtained during only the first or second of a series of ring tests performed on the same mice and it has therefore been assumed that these results need no correction. On the other hand, data concerning the full time course of cannabis action were obtained not only from the first two of these tests but also from the subsequent ones. Under these conditions, to provide some estimate of the adaptation by the animals to the test procedure, control mice were subjected to the same pattern of testing as drug-treated animals.

But this is not in fact an adequate control, since cannabis might well influence the process of adaptation. If it delays it, compared to the normal, then the effect

of cannabis will be underestimated. The time courses shown in Fig. 1, for example, are therefore only approximate.

The specificity of the ring test

The characteristic changes in behaviour on the ring induced by cannabis extract and described earlier can also be produced by chlorpromazine. Doses of both 1 and 2 mg/kg injected subcutaneously into mice at 30–32° C were effective. Sixty minutes after injection, a group of 6 mice treated with the former dose of chlorpromazine showed a mean immobility index of 37 ± 6, while a similar group treated with the latter dose showed a mean value of 49 ± 7. On the other hand, barbitone, which like cannabis and chlorpromazine can depress spontaneous activity, does not elicit the same type of behaviour on the ring as that induced by cannabis. Subanaesthetic doses of barbitone (50 to 150 mg/kg s.c.) had no significant effect on immobility indices measured at 30–32° C, 70 min after injection. Only doses of barbitone that were effective in abolishing the righting reflex (250 to 300 mg/kg s.c.) raised immobility indices significantly above those of saline-treated mice. In sharp contrast to the effect of cannabis, these increases were accompanied by a gross rise in the incidence of falls from the ring. The mice made no apparent effort to stay on the ring and the length of time passing before a fall occurred was governed by the way in which the animals were initially placed on the ring. For barbitone, the dose-response curve is steep; 200 mg/kg was ineffective while 250 mg/kg produced a mean immobility index of 99 ± 1 in a group of 4 mice.

Constituents of cannabis extract active in raising the immobility index

Δ^1-THC and Δ^1-THD are both effective in raising the immobility indices of mice at 30–32° C. Intraperitoneally, effective doses of Δ^1-THC ranged from 10 mg/kg upwards (Table 3): a dose of 5 mg/kg was not detectably active. For all effective doses of Δ^1-THC, the onset of peak effect defined above occurred within 30 min of injection. Both the peak effect and the duration of action were dose-related. Figure 3 shows log dose-response curves for cannabis extract and Δ^1-THC. The curves were plotted with data obtained 60 min after intraperitoneal injection of the drugs and included in Tables 1 and 3. The curves are approximately parallel and

TABLE 3. *Effect of Δ^1-tetrahydrocannabinol (Δ^1-THC) on the mean immobility index* (±S.E.M.) *of groups of mice at 30–32° C measured at several intervals after injection of the drug (i.p.)*

Experiment	Dose (mg/kg)		Mean immobility index (±S.E.M.) at 30, 60, 120, and 240 min after injection			
	Δ^1-THC	Tween	30	60	120	240
a	5	10	10±3 (6)	28±8 (6)	37±7 (6)	37±6 (6)
	10	20	48±6 (6)*	—	—	—
	15	30	64±6 (6)*	58±9 (6)*	43±6 (6)	34±5 (6)
	20	40	51±7 (6)*	—	—	—
	—	60	13±3 (6)	20±5 (6)	35±5 (6)	39±7 (6)
b	50	100	66±4 (21)*	70±2 (6)*	67±6 (6)*	55±8 (6)*
	—	100	12±5 (6)	9±3 (6)	20±3 (6)	34±3 (6)

Differences at each time after injection between drug and Tween treatments were evaluated by Student's *t* test. In both experiments, the comparisons were made between one or more drug treatments and a Tween treatment in which the dose of Tween administered was equal to or greater than that used in the drug treatments. Significant differences ($P<0.05$) are denoted by asterisks. The digits in parentheses denote the number of mice used. Mice were usually tested 4 times but in experiment b, 15 mice were tested only 30 min after injection.

show the relative potency of Δ^1-THC and cannabis extract to be between 10 and 20. Performance of a 2+2 symmetrical dose analysis to provide an estimate of the limits of error of this value was precluded for these data (see Fig. 3) by the occurrence of large differences between the variances of mean indices and by the non-linearity of the log dose-response relationships.

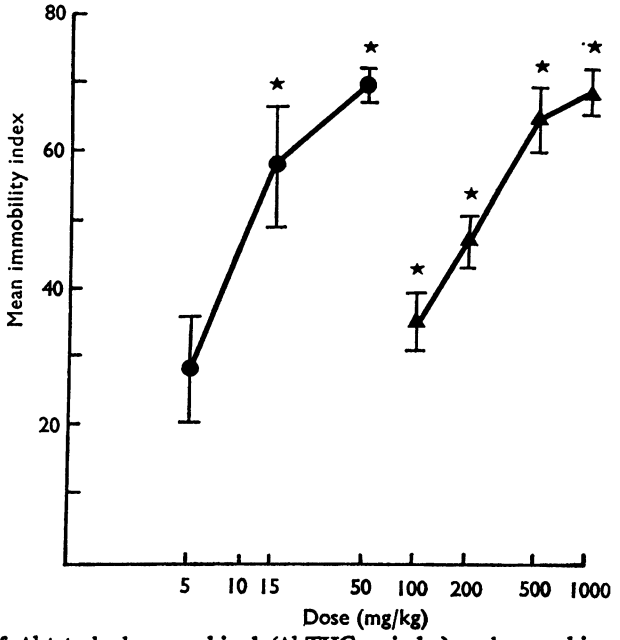

FIG. 3. Effect of Δ^1-tetrahydrocannabinol (Δ^1-THC; circles) and cannabis extract (triangles) on the immobility index (±S.E.M.) of mice measured at 30 to 32° C, 60 min after injection (i.p.). The data shown here also appear in Tables 1 and 3. Asterisks denote significant increases in the immobility index ($P<0.05$) compared to appropriate Tween 80 controls.

Preliminary experiments in which Δ^1-THC was injected i.v. at room temperature, showed that the intravenous threshold dose lies between 0·5 and 1·0 mg/kg.

Only a few experiments were performed with Δ^1-THD. The results obtained indicate that Δ^1-THD is less effective than Δ^1-THC in raising the immobility index. It was found that Δ^1-THD was effective in raising the index at doses ranging upwards from 13 mg/kg. Thirty min after the injection of this dose, the mean index of a group of 9 mice was 24 ± 4. Fifteen minutes after the administration of either 50 or 165 mg/kg, the mean indices of two groups of 8 mice were respectively 49 ± 6 and 64 ± 6. The solutions were injected i.p. at 30–32° C and contained 12 parts of Tween 80 to 1 part of Δ^1-THD by weight. The control experiment showed that even at a dose of 2 g/kg, Tween 80 did not produce a detectable rise in the immobility index: 15 min after the injection of this dose, the mean index of a group of 6 mice was 11 ± 4.

Finally, cannabidiol had no detectable effect when injected intraperitoneally in doses up to 100 mg/kg.

Discussion

Doses of cannabis effective in raising the immobility index elicited behavioural effects which may be summarized as (i) immobility of the animal and (ii) tolerance

of abnormal postures. The mice so affected remained arousable and retained both their agility and the righting reflex. This pattern of behaviour has been reported by other workers to follow treatment of a wide range of species with cannabis (Fraenkel, 1903 ; Loewe, 1946 ; Scheckel, Boff, Dahlen & Smart, 1968 ; Grunfeld & Edery, 1969 ; Lipparini, Scotti de Carolis & Longo, 1969 ; Carlini, Hamaoui, Bieniek & Korte, 1970a ; Carlini, Santos, Claussen, Bieniek & Korte, 1970b ; Christensen, Freudenthal, Gidley, Rosenfeld, Boegli, Testino, Brine, Pitt & Wall, 1971), and is generally referred to as the 'cataleptic' effect of the drug. Indeed it corresponds well with the many descriptions of experimental catalepsy that appear in the literature (e.g. Courvoisier, Ducrot & Julou, 1957 ; Munkvad, Pakkenberg & Randrup, 1968). The intermittent nature of the action of cannabis described in this paper has also been reported elsewhere (Grunfeld & Edery, 1969 ; Lipparini *et al.*, 1969).

Although the ring test provides a measure of the cataleptic effect of cannabis it is not a specific bioassay of cannabis nor a specific measure of catalepsy. Chlorpromazine is effective in raising the immobility index, eliciting behavioural changes on the ring apparatus indistinguishable from those produced by cannabis. Any treatment which merely depressed spontaneous activity should also raise the immobility index. Simple repetition of the test produces a rise in the index, probably due to an increasing familiarity of the mice with the environment provided by the experimental procedure. Hypothermia, by depressing spontaneous activity, might also influence the immobility index. It was found, however, that although the data obtained do not rule out an effect of hypothermia on the duration of action of cannabis, hypothermia did not significantly affect the peak effects of the crude extract on the immobility index.

The ring test does however possess some specificity: drugs which severely impair the agility of mice even if they also induce catalepsy or depress spontaneous activity can be distinguished from cannabis by the ring test. This point is illustrated by the experiment described earlier in which it was shown that barbitone becomes effective in raising the immobility index only at doses producing anaesthesia.

Despite the undesirably wide limits of error of the ring test, a defect it shares with other assays of cannabis that are based on behavioural effects of the drug, the test does offer certain advantages. First, although not completely specific, it is probably more specific as an assay of cannabis than other assays based on the cataleptic effect of the drug (Grunfeld & Edery, 1969 ; Carlini *et al.*, 1970b). In contrast to the ring test, these tests are unlikely to detect drug-induced changes in agility. Second, some assays of cannabis (Walton, Martin & Keller, 1938 ; Grunfeld & Edery, 1969) provide only quantal data since, unlike the ring test, they rely upon the arbitrary division of the effect of the drug on behaviour into distinct stages. Next, the ring test is convenient as a routine assay since untrained animals are used, and since each test lasts only five minutes. Another useful feature of the ring test is that it exploits an effect produced by cannabis in a wide range of species, making the assay potentially useful in comparative studies of the pharmacology of the drug. Also, mice are handled only briefly, ensuring minimal external interference with drug-induced changes. Finally, the method is relatively objective, relying upon the observation of easily recognizable changes in behaviour.

Both Δ^1-THC, which is generally believed to be the main centrally active constituent of cannabis and Δ^1-THD, were several times more effective than cannabis

extract in producing increases in the immobility index. The doses of Δ^1-THC found to raise the immobility index are also known to produce other behavioural changes in mice (Garriott, King, Forney & Hughes, 1967; Holzman, Lovell, Jaffe & Freedman, 1969; Grunfeld & Edery, 1969; Carlini *et al.*, 1970a; Carlini *et al.*, 1970b; Dewey, Harris, Howes, Kennedy, Granchelli, Pars & Razdan, 1970; Welch, Welch, Messiha & Berger, 1971). Because of the wide limits of error obtained in the ring test it was not possible to assess accurately the relative contributions made by Δ^1-THC and Δ^1-THD towards the effects of cannabis extract on the immobility index. However, since Δ^1-THC forms about 1/16th of the extract by weight, the finding that the relative potency of Δ^1-THC and cannabis extract is between 10 and 20, suggests that Δ^1-THC is the agent chiefly responsible for the effect of cannabis on the immobility index. This is supported by two other observations: first Δ^1-THD is less potent than Δ^1-THC in its effect on the immobility index, and second, cannabis extract contains approximately two parts of Δ^1-THC to one part of Δ^1-THD by weight.

Cannabidiol had no detectable effect on the immobility index even when administered at a dose ten times the threshold dose of Δ^1-THC. However, even if it does not contribute directly towards the effect of cannabis on the immobility index, cannabidiol may act indirectly by inhibiting the metabolism of Δ^1-THC (Jones & Pertwee, 1972).

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