Br. J. Pharmac. (1972), **44**, 250–261.

Effect of cannabis and certain of its constituents on pentobarbitone sleeping time and phenazone metabolism

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Summary

1. Cannabis extract prolonged sleeping time in mice in a thermally neutral environment (30–32° C) in which hypothermia does not occur. The prolongation was dose related, just detectable at 50 mg/kg, and 4-fold at 500 mg/kg.

2. Under these conditions, ether sleeping time was not prolonged.

3. Cannabis extract inhibited the aerobic metabolism of phenazone by a microsome-rich 9,000 *g* supernatant of mouse liver homogenate capable of nicotinamide adenine dinucleotide phosphate (NADPH) generation.

4. Δ^1-Tetrahydrocannabinol (Δ^1-THC) prolonged pentobarbitone sleep and inhibited phenazone metabolism, but its action was limited, and could not account for the effect of the extract. The carotenes and water-soluble fractions of the extract were inactive on pentobarbitone sleep.

5. Cannabidiol was strongly active by both tests; *in vivo* 39·8 μM/kg (12·5 mg/kg) prolonged sleep by 190%, and *in vitro* 12·7 μM inhibited phenazone metabolism 20%. These actions were dose related, and could account for the effect of the extract.

6. The prolongation of pentobarbitone sleep by cannabis extract in a dose of 200 mg/kg, intraperitoneally, was maximal when given 30 min before the pentobarbitone, still present at 3 h, but undetectable at 24 hours. No phase of enhanced metabolism at 24 or 48 h after single cannabis injection was detected.

7. It is concluded that cannabis extract inhibits microsomal activity of mouse liver, chiefly by virtue of its cannabidiol content. It is probable that cannabis consumption by man could lead to altered disposal of many other drugs, used in medicine or otherwise.

Introduction

The observation, originally made by Loewe (1944), that cannabis prolongs sleep induced by certain barbiturates, has been confirmed both for cannabis extracts (Bose, Saifi & Bhagwat, 1963; Miras, 1965; Garrattini, 1965) and for Δ^1-tetrahydrocannabinol (Δ^1-THC) (Kubena & Barry, 1970; Forney, 1971). The experiments described in this paper are concerned both with the mechanism of action of this effect in mice and with the constituents of cannabis responsible for it. The possibilities that the effect was due to hypothermia or to a central effect potentiating barbiturate hypnosis were first investigated. The effect of cannabis extract on phenazone metabolism by a microsome-rich fraction of a homogenate of mouse liver was then examined. Finally the relative effects on sleeping time and on phenazone

metabolism have been compared for the extract and a number of its constituents. A preliminary account of the experiments on sleeping time has been published (Gill, Paton & Pertwee, 1970), and a demonstration was made to the British Pharmacological Society at its meeting on 16th September, 1971.

Methods

Drugs were administered to mice either intraperitoneally or subcutaneously. The mice used were non-fasted, adult male mice weighing 20–30 g supplied by A. J. Tuck. Cannabis was supplied as an ethanolic tincture (BPC 1949), prepared commercially by extracting with cold ethanol the leaves and flowers of *Cannabis sativa* of Pakistani origin, evaporating the solvent and dissolving the resinous residue in fresh ethanol. The residue has been found (Dr. E. W. Gill, personal communication) to contain 6·4% by weight of Δ^1-THC, 3·4% by weight of the n-propyl analogue of Δ^1-THC, Δ^1-tetrahydrocannabidivarin (Δ^1-THD: Gill, 1971) and 3·6% by weight of cannabidiol. (The earlier figures quoted by Gill *et al.* (1970) for Δ^1-THC and Δ^1-THD refer to the yield of material when isolated from the residue of the tincture.) The crude cannabis resin, natural amyl Δ^1-THC, carotenes, cannabidiol (CBD) and the petrol-ether-soluble and insoluble fractions of cannabis resin used in the experiments described in this paper were each prepared for administration by dispersion in a mixture of Tween 80 and a 0·9% w/v 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 the carotenes extracted from the petrol-ether-soluble fraction of crude resin, and to Professor R. Mechoulam for a sample of cannabidiol. The solutions of crude cannabis in the earlier experiments contained one part of drug to 1·6 of Tween 80, in the later experiments 2·4 parts of drug to one part Tween 80, by weight; the solutions of the materials derived from cannabis contained two parts of Tween 80 to one part of material by weight. Control injections were made with solutions containing appropriate amounts of Tween 80 in 0·9% w/v NaCl solution.

Sleeping time experiments on groups of five to ten mice were conducted in a quiet room maintained at a temperature of 30–32°C. In pilot experiments, a dose of 25 mg/kg pentobarbitone given intraperitoneally did not produce sleep; 50 mg/kg produced sleeping times ranging from 12 to 65 min for individual mice, giving means for a group of the order of 30–35 minutes. When given subcutaneously, slightly larger doses are required and the following mean sleeping times were obtained: 50 mg/kg, 10 min; 63 mg/kg, 43 min; 100 mg/kg, 75 minutes. The dose of pentobarbitone chosen for most experiments (50 mg/kg i.p.) was such as to induce sleep in all animals. With animals at room temperature, pentobarbitone (100 mg/kg i.p.) produced a mean sleeping time of 93 min (49–146 min); and 600 mg/kg subcutaneously a sleeping time of 118 min (53–180 min). Pentobarbitone was used as the sodium salt dissolved in 0·9% w/v NaCl solution and doses quoted are in terms of this salt. Ether was injected dissolved in olive oil. Intraperitoneal injections of ether or pentobarbitone were adjusted to a volume of 10 ml/kg body weight. Sleeping time was taken to be the difference between the time of injection of pentobarbitone or ether and the time at which a mouse permanently recovered its righting reflex. Mice were considered to have positive righting reflexes if they consistently regained a position with all four feet on the floor within 10 s of being rolled on to their backs. Materials being screened for an effect on sleeping time were administered at a specific interval, usually 30 min before the pentobarbitone or ether. Once

injected with the hypnotic, mice were placed on their backs. After an animal had rolled back from this position on to its belly, several attempts were made to place it once more on its back in order to test whether or not the righting reflex had been permanently restored.

The drug metabolizing capacity of mouse liver was measured by following the metabolism of phenazone in incubation mixtures containing hepatic microsomes and soluble enzymes. The methods used were based on those recommended for rats by Jóhannesson, Rogers, Fouts & Woods (1965) and by Neugebauer, Splinter, Häfke, Kober, Schirlitz & Klinger (1969). The pathways of phenazone metabolism in mice have been studied by Yoshimura, Shimeno & Tsukamoto (1968).

Hepatic microsomes and soluble enzymes were obtained from pooled samples of liver tissue which was removed from ten to fifteen mice and washed immediately in ice-cold 1·15% aqueous KCl solution. Gall bladders were left intact and excluded from the samples. The liver was chopped up and resuspended in 1·15% aqueous KCl solution, each gramme of liver being mixed with 2 ml of the ice-cold solution. The liver was homogenized at 0–5° C in a Potter-Elvehjem homogenizer (clearance 0·08 mm) having a glass mortar and a Teflon pestle. Homogenization was carried out by passing the pestle up and down 6 times as gently as possible. The resulting homogenate was then centrifuged for 20 min at 9,000 *g*, the temperature being kept at 0–5° C. The supernatant fraction contained 44·1 (±1·75 S.E.) mg protein per ml as determined by the method of Lowry, Rosebrough, Farr & Randall (1951).

Incubations with the 9,000 *g* supernatant were conducted aerobically in 25 ml conical flasks which were shaken in a water bath at 37° C. Each flask contained 1·5 ml of supernatant and 0·9 ml of 0·2 M Tris acid maleate-NaOH buffer of pH 7·5 (v. Long, 1961) containing 40 μmol of nicotinamide, 10 μmol of magnesium sulphate, 0·22 μmol of NADP, and 10 μmol of glucose-6-phosphate. Flasks were incubated in batches of fourteen. Of these half were control flasks containing 10 μl of ethanol while the remainder were test flasks containing test material dissolved in 10 μl ethanol. After preincubation for 5·5 min, 2·5 μmol phenazone were added dissolved in 0·1 ml water to all but one test and one control flask. The latter acted as blanks and contained water instead of the phenazone solution. The total volume of mixture in each flask was 2·51 ml.

Incubations were stopped by the addition of 10 ml 7% aqueous trichloroacetic acid solution. The latter was added to one test flask and one control flask before the addition of the phenazone. The remaining (twelve) flasks were incubated with phenazone for 30 min and the reaction was then stopped. After standing in ice for 30 min the protein precipitated by the trichloroacetic acid was centrifuged down and the determination of unchanged phenazone in the resultant supernatant was carried out according to the method of Brodie, Axelrod, Soberman & Levy (1949).

Results

Prolongation by cannabis of pentobarbitone-induced sleep in mice in a thermally neutral environment

The first experiments showed that prolongation of pentobarbitone sleeping time in mice by cannabis extract is observed even if the mice are exposed during the experiment to an environmental temperature of 30–32° C, known to be the thermally neutral zone for the mouse (Herrington, 1940). Table 1 summarizes the data ob-

tained with different doses of cannabis given at intervals between 1 min and 48 h before the injection of pentobarbitone. The prolongation was detectable with 50 mg/kg of cannabis, if given 1 min before the pentobarbitone, when a prolongation of 56% was obtained; but the effect was insignificant at this dose with an interval of 30 minutes. Taking the 30 min interval, which at higher doses corresponded approximately to the interval for maximum effect, Fig. 1 shows how the percentage prolongation increases steadily with rising doses of cannabis. In a few experiments with the animals at room temperature, sleep occurred after administration of cannabis (500 mg/kg, i.p.), followed by pentobarbitone (300 mg/kg, s.c.) 50 min later.

Table 1 gives the results of experiments on the time course of cannabis action and Fig. 2 the same results expressed as percentage prolongation. With a threshold dose, as mentioned above, the effect was only significant when cannabis was injected just before the pentobarbitone. With a higher dose, the effect was greater with the 30 min interval, declined to about one-half of this value with a 3 h interval, and was undetectable at 24 hours. Since it was possible that inhibition of barbiturate metabolism might be succeeded by a period of induction, the experiment was repeated with an extension of the interval to 48 h; but there was no significant difference between 24 h, 48 h, and control values.

The temperature of 30–32° C had been chosen as being in the thermal neutral zone of the mouse. It was verified in one experiment that this was correct for our animals. A dose of extract (25 mg/kg, i.v.) sufficient to produce a maximum fall of 2·5° C at room temperature, was given to a group of six mice kept at 30–32° C. Their mean rectal temperature at 30 min (the normal time of peak effect for this

TABLE 1. *Effect of cannabis extract on pentobarbitone* (50 *mg/kg, i.p.*) *sleeping time in mice*

Drug	Pretreatment Dose (mg/kg)	Time before pentobarbitone injection	Mean sleeping time (min ± S.E.M.)	(No. of animals)	*P*
		Experiment 1			
Cannabis extract	50	30 min	38·47 ± 5·05	(9)	>0·1
		3 h	38·78 ± 4·39	(9)	>0·1
		24 h	28·0 ± 5·08	(9)	>0·1
Cannabis extract	200	30 min	84·17 ± 13·38	(9)	<0·01
		3 h	56·17 ± 3·79	(9)	<0·01
		24 h	28·28 ± 4·43	(9)	>0·1
Tween control	320	30 min	34·19 ± 4·67	(9)	—
		3 h	33·31 ± 5·07	(9)	—
		24 h	30·92 ± 5·38	(9)	—
		Experiment 2			
Cannabis extract	50	1 min	46·94 ± 4·68	(8)	0·02*
Cannabis extract	200	1 min	57·13 ± 7·30	(8)	<0·01*
		30 min	66·69 ± 11·22	(8)	<0·01*
		24 h	43·16 ± 5·75	(8)	>0·05*
		48 h	35·81 ± 6·77	(8)	>0·1
Tween control	320	30 min	30·16 ± 4·38	(8)	—
		48 h	30·72 ± 4·22	(8)	—
Saline control	10 ml/kg	30 min	31·22 ± 5·26	(8)	>0·1*

* Compared to 30 min Tween control. To avoid systematic error particularly due to diurnal variation in the activity of the mice, the order of injections on successive days in the two experiments was arranged according to 9 × 9 and 8 × 8 Latin Square designs. The effects of the different doses of extract were compared statistically with those of the Tween controls for the same preinjection interval where available, and otherwise with the 30 min Tween control.

route) was 39·1° C, and did not differ significantly from that of an untreated group of five mice (39·1° C) or a Tween-injected group of six mice (39·5° C).

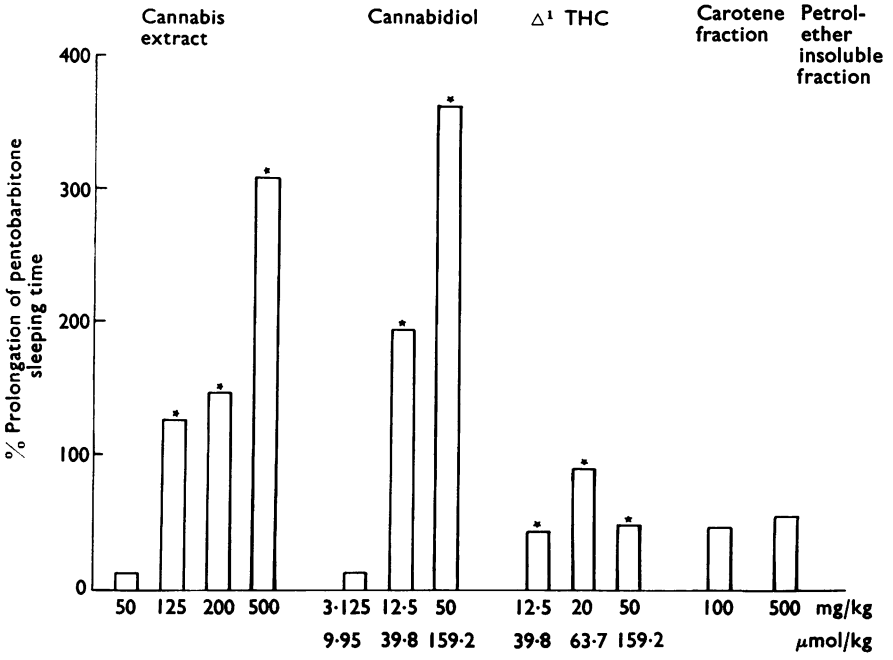

FIG. 1. Effect on pentobarbitone sleeping time in mice of cannabis extract, cannabidiol, Δ^1-THC, and the carotene and petrol ether-insoluble fractions of the extract. Data from Tables 1 and 4 expressed as percentage prolongation. Asterisks denote significant increases in sleeping time ($P<0.05$).

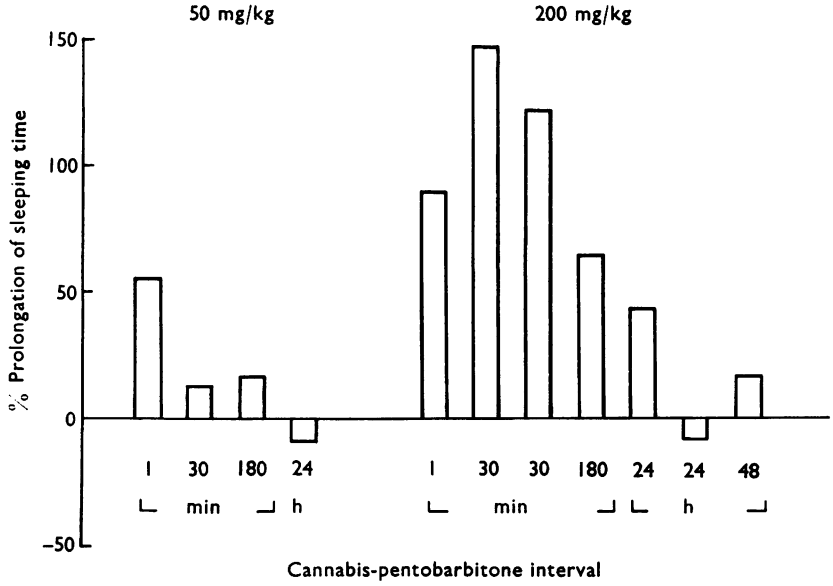

FIG. 2. Effect of varying the interval between cannabis extract and pentobarbitone injections on sleeping time prolongation. Data from Table 1 expressed as percentage prolongation.

Effect of cannabis on ether sleeping time

To investigate whether a central effect of cannabis could potentiate the anaesthetic effect of pentobarbitone in some way independent of metabolism, a non-metabolized anaesthetic was required. Barbitone itself was tested, but the sleeping times were too long (80 to >140 min) to be suitable, and also too variable to provide a satisfactory test. It was found, however, that ether (given intraperitoneally dissolved in olive oil) in a dose in the range 2·2–2·5 g/kg (29·7–33·7 mM/kg) gave sleeping times of 12–22 minutes. The dose could not be raised further without killing many of the animals. Even with the dose used in the experiment of Table 2, three animals died in each of the two ether treated groups, and are omitted from the table. It was also found that ether in olive oil and cannabis in Tween 80 NaCl solution could not be given together intraperitoneally, since the Tween solution itself, in some way not further investigated, enhanced the acute lethality of ether. Accordingly, cannabis was given subcutaneously, although a larger dose was needed to produce its characteristic effect. Table 2 gives the results of the experiment. Under conditions where pentobarbitone sleeping time was increased by 71%, the ether sleeping time was unaffected.

Effect on a preparation of mouse liver containing microsomal enzymes

In preliminary experiments using the conditions recommended by Neugebauer *et al.* (1969) for rat liver, the rate of metabolism of phenazone was linear up to 30 min incubation time, but the fraction metabolized in 30 min did not amount to more than about 15–20%. When the concentration of phenazone was halved, it was possible to obtain a linear rate of metabolism over 30 min of up to 32% of the phenazone present. It was also found in preliminary experiments that ethanol in a concentration of 67·4 mM did not impair metabolism. The tests were made, therefore, using the cannabis extract dissolved in ethanol, and incubated for 30 min with

TABLE 2. *Effect of cannabis extract, given subcutaneously, on sleeping time due to pentobarbitone or diethyl ether given intraperitoneally*

Pretreatment				Mean sleeping time		
Drug	Dose (mg/kg)	Hypnotic agent	Dose (mg/kg)	(min±S.E.M.)	(No. of animals)	*P*
Cannabis extract	1,000	Pentobarbitone	50	59·03±6·89	(9)	<0·01
Tween control	400	Pentobarbitone	50	34·58±4·0	(9)	—
Cannabis extract	1,000	Ether	2,342	15·61±1·82	(9)	>0·1
Tween control	400	Ether	2,342	13·50±0·87	(9)	—

TABLE 3. *Effect of cannabis extract on metabolism of phenazone by a microsome-rich fraction of a homogenate of mouse liver*

Treatment		Mean phenazone concentration±S.E.M.		
Drug	Concentration (mg/l.)	Initial (μmol/g liver)	Final	*P*
Cannabis extract	40	2·737±0·174 (2)	2·005±0·054 (5)	0·047
Ethanol control	3,100		1·846±0·041 (5)	—
Cannabis extract	200	3·017±0·031 (2)	2·707±0·061 (5)	<0·001
Ethanol control	3,100		2·194±0·042 (5)	—

The initial phenazone concentrations were measured on one test flask and one control flask.

the enzyme preparation. If there is any latency in uptake of the active principles of cannabis by the preparation, as there is in its other actions, the size of the described effects may be somewhat underestimated.

Table 3 and Fig. 3 summarize the results with cannabis extract. A significant inhibition of 17·5% was found at 100 μg extract per flask, and 67% inhibition at 500 μg extract per flask.

Constituents of cannabis extract active on sleeping time and on microsomal enzymes

Δ^1-THC is also able to prolong barbiturate sleeping time under thermally neutral conditions (Table 4) and to inhibit phenazone metabolism (Table 5). But the effect of Δ^1-THC was far from accounting for the activity of the extract. Its dose-response curve was flatter than that of extract in both tests, and its prolongation of sleeping time appeared to reach a maximum with a prolongation of less than 100%. Further, although potency comparisons are difficult because of the lack of parallelism of the dose-response curves of Δ^1-THC and of extract, the potency ratios of Δ^1-THC to extract are of the order of 2–5, whereas if Δ^1-THC were solely responsible, the potency ratio should be about 15. The carotene fraction and the petrol-insoluble fraction of the extract were wholly inactive on barbiturate sleeping time (Fig. 1 and

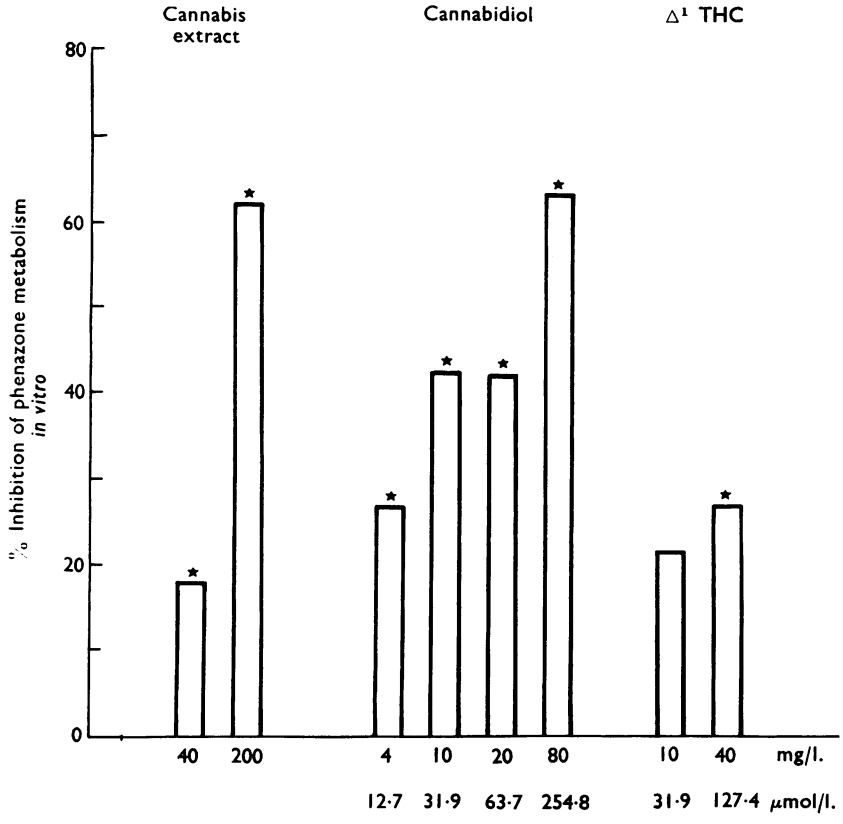

FIG. 3. Inhibition of phenazone metabolism by 9,000 *g* supernatant of mouse liver homogenate. Data from Tables 3 and 5 expressed as percentage inhibition. Asterisks denote a significant inhibition of phenazone metabolism ($P<0.05$).

Table 4). But cannabidiol (CBD) was highly active in both tests, and the activity was strongly dose-dependent. On sleeping time it was about 20 times more active than the extract. With phenazone metabolism, the dose-response curves obtained were not parallel, and the relative potency varied from 12 at threshold effect to 2–3 at high effect. Insufficient experiments were done to establish reliable potency relations between extract, Δ^1-THC and cannabidiol, but it was clear that cannabidiol was much more active than Δ^1-THC, and that in both tests it could be responsible for the bulk, if not all, of the activity of cannabis extract.

TABLE 4. *Effect of cannabis extract and of its constituents on pentobarbitone (50 mg/kg, i.p.) sleeping time in mice*

Pretreatment Drug	Dose (mg/kg)	Mean sleeping time (min±S.E.M.)	(No. of animals)	*P*
Cannabis extract	125	110·42±22·75	(5)	0·028
	500	199·20±53·51	(5)	<0·05*
Δ^1 THC	12·5	70·2± 4·70	(5)	0·038
	50	72·95± 4·62	(5)	0·025
Tween control	200	48·73± 7·16	(5)	—
Δ^1 THC	20	90·69± 7·62	(5)	<0·001
Tween control	40	47·37± 2·29	(5)	—
Carotene fraction	100	80·75± 8·88	(5)	0·094
CBD	50	255·70±12·08	(5)	<0·001
Tween control	200	55·08±10·17	(5)	—
CBD	3·125	51·97± 4·79	(5)	>0·1
CBD	12·5	136·22±16·08	(5)	<0·001
Tween control	25	46·07± 8·41	(5)	—
Petrol ether–insoluble residue	500	52·7± 3·99	(5)	0·093
Petrol ether–soluble residue	500	>201·4	(5)	—
Tween control	200	43·35± 2·81	(5)	—

* In this test, the ratio of its variance to that of control was $F=55·9$. Accordingly Cochran's (1964) approximation to the Behrens-Fisher test was used.

TABLE 5. *Effect of Δ^1-tetrahydrocannabinol (Δ^1-THC) and cannabidiol (CBD) on metabolism of phenazone by a microsome-rich fraction of a homogenate of mouse liver*

Treatment Drug	Concentration (mmol/l.)	Mean phenazone concentration±S.E.M. Initial (μmol/g liver)	Final (μmol/g liver)	*P*
Δ^1 THC	0·0319	2·892±0·028 (2)	2·294±0·103 (5)	>0·1
Ethanol control	67·39		2·130±0·037 (5)	—
Δ^1 THC	0·127	2·652±0·023 (2)	2·016±0·037 (5)	0·005
Ethanol control	67·39		1·781±0·050 (5)	—
CBD	0·0127	2·834±0·024 (2)	2·260±0·018 (5)	0·002
Ethanol control	67·39		2·050±0·044 (5)	—
CBD	0·0319	2·899±0·118 (2)	2·391±0·014 (5)	<0·001
Ethanol control	67·39		2·016±0·021 (5)	—
CBD	0·0637	2·768±0·069 (2)	2·317±0·025 (4)	<0·001
Ethanol control	67·39		1·988±0·029 (5)	—
CBD	0·255	2·968±0·024 (2)	2·617±0·029 (5)	<0·001
Ethanol control	67·39		2·011±0·025 (5)	—

Effect of cannabis on the acute lethality of pentobarbitone

Prolongation of the action of pentobarbitone by cannabis would affect the acute lethality of the barbiturate in so far as a significant metabolism of pentobarbitone occurs during the period before death. Table 6 gives the results of two experiments, in which doses of 100 mg/kg and 150 mg/kg pentobarbitone were given intraperitoneally to mice which also received either 1 g/kg cannabis extract or the corresponding Tween excipient 30 min earlier. There was no significant difference between the acute mortality rates at the two dose levels, but some indications of earlier death with cannabis treated animals. With the higher dose of barbiturate, the deaths occurred at times after injection of barbiturate up to 20 min in the cannabis group, and up to 33 min in the Tween control group. Any effect of cannabis on the acute intraperitoneal LD50 of pentobarbitone must be relatively small.

Discussion

The main object of this investigation was to discover whether the prolongation of barbiturate action by cannabis extract represented an effect on barbiturate metabolism or not. Hypothermia is known to prolong barbiturate sleeping time (Raventós, 1938) and since cannabis given to animals at room temperature can produce substantial and sustained falls in body temperature, at least up to 8° C (Gill *et al.*,1970), this might have accounted for the effect in full. It is likely that, in experiments at room temperature, hypothermia has a contributory action. But the presence of the effect under conditions where normal body temperature is maintained, together with the failure of cannabis to potentiate sleeping time produced by a hypnotic, ether, which is not metabolized, made it probable that the drug was interfering with metabolism of the barbiturate by inhibiting the activity of liver microsomes. This was supported by the finding that cannabis extract inhibited phenazone metabolism by the 9,000 *g* supernatant from a mouse liver homogenate.

It seems probable that the metabolic effects are specifically on the microsomal systems of the liver, rather than on the soluble enzymes, since there is evidence (Neugebauer *et al.*, 1969) that both pentobarbitone and phenazone are degraded by microsomal enzymes, and we have used a microsome-rich preparation containing a system generating NADPH under aerobic conditions for our *in vitro* studies.

The question arises whether this inhibition is adequate to account for the effect on barbiturate sleeping time. Folz, Fentiman, Leighty, Walter, Drewes, Schwartz, Page & Truitt (1970) showed, in rats, that 13% of a dose of Δ^8-THC was found in the liver 30 min after injection. If a similar proportion of cannabis extract passes to the liver in the mouse, then, for example, after a dose of 4 mg to a 20 g mouse (200 mg/kg), which is sufficient to prolong sleeping time by 150%, 0·52 mg of extract

TABLE 6. *Effect of cannabis extract* (1 *g/kg s.c.*) *on the acute lethality of pentobarbitone given intraperitoneally*

Pretreatment	Pentobarbitone Dose (mg/kg)	Deaths
Cannabis extract	100	0/10
Tween control	100	1/10
Cannabis extract	150	7/10*
Tween control	150	8/10

* Three delayed deaths not included.

will be present in its liver ;; for a liver weight of 1·3 g (Webster & Liljegreu, 1955) this gives a concentration of cannabis in the liver of 400 μg/g. For comparison, the addition of 100 μg extract to 2·5 ml of homogenate, representing 0·76 g of liver, gives a concentration of 40 μg/ml in the flask, or if the extract is taken up by the homogenate, up to 132 μg cannabis per gramme of liver ; this produced an inhibition of phenazone metabolism of 17% ; and 500 μg extract, corresponding to a concentration of 200 μg/ml or up to 658 μg/g liver, produced an inhibition of 67%. The relation between the inhibition of microsomal enzymes and prolongation of sleeping time must be complex, since part of the termination of barbiturate action will involve the redistribution of the drug. But the effect of the extract on metabolism is evidently likely to contribute to, or even account fully for, the effect on the sleeping time. This conclusion is supported by finding a correspondence between the prolongation of pentobarbitone induced sleep *in vivo* and the *in vitro* actions on phenazone metabolism, when testing different doses of the relatively inactive Δ^1-THC or of the highly active cannabidiol.

Our experiments have confirmed in full Loewe's observation that cannabidiol was particularly effective in prolonging barbiturate sleeping time, and provisionally, it appears that cannabidiol can account for most, if not all, of this effect of the extract by its action in inhibiting microsomal enzymes. We have not, however, been able to test a light petroleum-soluble fraction which was free of Δ^1-THC and cannabidiol, so that other related active substances may be present.

The ability to prolong barbiturate sleeping time has been shown for Δ^3-THC and pyrahexyl (Garriott, King, Forney & Hughes, 1967), 7-OH-Δ^1-THC (Truitt, 1970), the dimethyl heptyl and methyl-octyl derivatives of Δ^3-THC (Dagirmanjian & Boyd, 1962) and certain 3-OH-6-dibenzopyran derivatives (Garriott, Forney, Hughes & Richards, 1968), so that, although these experiments were not made under conditions in which normal body temperature was maintained, it is likely that the property is, with more or less potency, shared by many cannabinoids. The lipophilic nature of the active principles of cannabis suggests that such an inhibition of enzymic activity might be relatively unspecific, but the much higher activity of cannabidiol compared to Δ^1-THC shows that structural features are important. Since cannabidiol was very active *in vivo* at 12·5 mg/kg, and *in vitro* at 4 μg/ml (12·7 μM), it is one of the stronger metabolic inhibitors and is about as potent as SKF 525A. The action of Δ^1-THC also shows specificity since it has been found not to inhibit monoamine oxidase (Schildkraut & Efron, 1971) or catecholamine uptake (Maitre, Staehelin & Bein, 1970) and its properties *in vitro* do not suggest any inhibition of cholinesterase (Gill & Paton, 1970). Δ^1-THC is a substrate for microsomal hydroxylation (Burstein, 1971). It may be that the phenolic character of the cannabinols and the phenolic and alcoholic hydroxyl groups of cannabidiol in particular, confer a specific affinity for some component of the hydroxylating system of the microsomes..

It should be noted that for prolongation of barbiturate sleep by Δ^1-THC other factors may be involved. Kubena & Barry (1970) showed that it prolonged barbitone as well as pentobarbitone sleep in rats, and concluded, since barbitone is not metabolized, that the effect involved a central potentiation by Δ^1-THC of the barbiturates. Sofia & Barry (1970) found that SKF 525A and Δ^1-THC produced a very considerable mutual potentiation of the prolongation of barbiturate sleep, and inferred that it was Δ^1-THC rather than a metabolite that was acting synergistically with

barbitone on the central nervous system. The interpretation of these experiments is not clear, since hypothermia could have occurred, with its own effects on metabolism, on renal excretion and on central nervous activity. But the curious difference in our own experiments between the effects of cannabis extract and cannabidiol on the one hand, and the limited prolongation achievable by Δ^1-THC on the other, does leave open the possibility of other mechanisms being involved.

There was no sign with cannabis extract, judging by the sleeping time results, that the period of impaired barbiturate metabolism is succeeded by enhancement of metabolism, such as occurs with SKF 525A, and is believed to represent induced microsomal activity (Kato, Chiesara & Vassanelli, 1964). Similarly Dewey, Kennedy & Howes (1970) found that five daily injections of Δ^1-THC in rats did not stimulate microsomal activity. Our experiments, however, have so far only used single doses, and it remains possible that induction may follow repeated administration of cannabis or cannabidiol, and may contribute to the now well established tolerance to the behavioural effects of cannabis in animals.

The existence of an activity of cannabis on microsomal enzymes has a significance beyond that of barbiturate and phenazone metabolism, since exposure to cannabis could lead to altered metabolism of many other drugs, not only those liable to abuse, such as other hypnotics, amphetamines, or alcohol, but also of many drugs used in medical treatment.

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(*Received October* 20, 1971)