Biochemical Pharmacology, Vol. 23, pp. 477–488. Pergamon Press, 1974. Printed in Great Britain.

EFFECT OF CANNABIS ON PENTOBARBITAL-INDUCED SLEEPING TIME AND PENTOBARBITAL METABOLISM IN THE RAT*

ALBERT J. SIEMENS, HAROLD KALANT, JATINDER M. KHANNA, JOAN MARSHMAN and GREGORY HO

Department of Pharmacology, University of Toronto, and
The Alcoholism and Drug Addiction Research Foundation, Toronto, Canada

(*Received* 28 *February* 1973; *accepted* 27 *July* 1973)

Abstract—The effects of two cannabis extracts with different cannabinoid compositions, as well as of pure Δ^1-tetrahydrocannabinol (THC), cannabinol (CBN) and cannabidiol (CBD), on pentobarbital metabolism were studied in the rat. Extract I, with high proportions of CBN and CBD relative to THC, when given by gavage 21·5, 40 or 63 hr before pentobarbital (30 mg/kg, i.p.), prolonged the sleeping time by 53, 42 and 21 per cent respectively. This effect was paralleled by decreases in the rate of disappearance of [^{14}C]pentobarbital from the blood, and of pentobarbital metabolism by liver microsomal preparations *in vitro*. Extract II, with low relative proportions of CBN and CBD, did not have any significant effect on pentobarbital metabolism or sleeping time. CBD alone, in the same dose as that given in Extract I, had very similar effects, while a dose of CBD equivalent to that given in Extract II had no effect. THC, CBN and CBD added to normal rat liver microsomes *in vitro* inhibited pentobarbital metabolism competitively, CBD being a much more potent inhibitor than THC and CBN. The CBD content may, therefore, be a significant factor in interactions between marijuana and other drugs.

SINCE MARIJUANA may be used in conjunction with drugs such as barbiturates, ethanol, amphetamines, opiates and potent hallucinogens,[1–4] it has become of interest to determine how and to what extent cannabis preparations interact with these other drugs. For example, cannabis extracts[5,6] and Δ^1-tetrahydrocannabinol (THC)[7–9] have been found to prolong barbiturate-induced sleeping time in rats and mice. Recently, Paton and Pertwee[10] demonstrated prolongation of pentobarbital-induced sleep in mice when pentobarbital was given at 30 or 180 min but not at 24 hr after single doses of either cannabis extract, THC or cannabidiol (CBD). These preparations also inhibited phenazone metabolism *in vitro* in mouse liver. The authors suggested that cannabidiol, rather than THC, was the ingredient of the cannabis extract primarily responsible for the effect on drug metabolism. In addition, Jones and Pertwee[11] suggested that CBD inhibited THC metabolism in mice *in vivo*.

These studies left a number of questions unanswered. Since the effect of cannabis on drug metabolism was studied with phenazone rather than pentobarbital as substrate,[10] it could not be concluded that the prolongation of pentobarbital sleep was caused by decreased rate of metabolism. Indeed, Paton and Temple[12] found that thiopental sleeping time was significantly prolonged in rabbits when measured 5 days after the last day of chronic administration of cannabis extract; but termination of thiopental anesthesia is known to depend on drug redistribution rather than on

* Supported in part by Grant NHG-606-25-2, National Health and Welfare, Canada.

metabolism. In addition, it seemed desirable to confirm *in vivo* as well as *in vitro* the striking finding of the role of CBD in the modification of drug metabolism.[10] Finally, because of known differences between rats and rabbits with respect to metabolism of cannabinoids,[13] it was of interest to study the above effects in rats.

The present study was undertaken to answer these questions. The results show that single doses of cannabis preparations, administered up to 63 hr before pentobarbital, prolong pentobarbital-induced sleep in rats and inhibit pentobarbital metabolism both *in vivo* and *in vitro*. The effects appear to be explainable almost entirely in terms of the CBD content of the cannabis extracts.

MATERIALS AND METHODS

Cannabis preparations. Two lots of marijuana were obtained from the Department of National Health and Welfare, Ottawa, Canada. Lot I had been grown in Canada in 1969, while lot II was of Mexican origin [NIMH Lot No. MS-11(2-PF-126)]. The cannabinoid compositions as determined by gas-liquid chromatography (GLC) with 3% OV-17 on Gas Chrom Q[14] were: lot I—THC, 0·17%; CBD, 1·670%; cannabinol (CBN), 0·425%; lot II—THC, 1·680%; CBD, 0·148%; CBN, 0·309%. The cannabigerol (CBG) content in both lots was too low to be determined accurately.

Cannabis extract was prepared by extracting 50 g of marijuana three times, for 12 hr each time, with a mixture of 400 ml of 95% ethanol and 200 ml of methanol. The solvents were evaporated *in vacuo* at 40°. The residue was redissolved in 200 ml of hexane and the solution was washed three times with 60 ml of 1% sodium bicarbonate and three times with 40 ml of distilled water. The solution was then dried over anhydrous sodium sulfate, the hexane was removed *in vacuo* at 35°, and the residue was dissolved in 100 ml of 95% ethanol for storage at 5°. For animal treatment, the ethanol was evaporated *in vacuo* and the residue was dissolved in sufficient olive oil to yield a preparation with a THC concentration of 2·5 mg/ml. This resulted in preparations with the following compositions: lot I—THC, 2·5 mg/ml; CBD, 5·85 mg/ml; CBN, 1·49 mg/ml; total residue, 20·83 mg/ml; lot II—THC, 2·5 mg/ml; CBD, 0·22 mg/ml; CBN, 0·46 mg/ml; total residue 6·55 mg/ml.

The cannabis extracts were administered by stomach tube in amounts providing a THC dose of 10 mg/kg (1 ml/250 g body wt). In other experiments, the pure cannabinoids, Δ^1-THC and CBD, were also administered orally in varying doses in the same volume of olive oil. Control rats received equivalent volumes of olive oil.

Animals. Male Wistar rats weighing 280–310 g were housed in group cages and were offered Purina chow and tap water *ad lib.* Rats were pretreated by gavage with the respective marijuana preparations, pure cannabinoids, or olive oil, at specific time intervals before the determination of pentobarbital-induced sleeping time, the rate of pentobarbital disappearance from the blood *in vivo*, and the metabolism of pentobarbital *in vitro*. The selected time intervals are indicated in the Results section.

Pentobarbital-induced sleeping time. All experiments were carried out between 10.00 a.m. and 3.00 p.m. in a quiet room at 23°. Pentobarbital sodium (3 mg/ml) in 0·9% NaCl, w/v, was given, i.p., at a dose of 30 mg/kg. The sleep induction time was defined as the time from the injection to the loss of the righting reflex (RR). Sleeping time was defined as the elapsed time between the loss and recovery of the RR. (An animal was considered to have demonstrated the RR if it was able to turn from its back onto all four limbs on three consecutive tries.) The investigator who

measured the time intervals was not informed of the identities of the control and treated groups. Immediately after the animal regained the RR, 200 μl of blood was taken from the tail tip and pentobarbital concentration in the blood was measured by GLC.[15]

Pentobarbital disappearance in vivo. Rats received either cannabis extract, lot I, or olive oil 21·5 hr before pentobarbital disappearance from the blood was determined. Animals were anesthetized with ether and the right jugular vein was exposed through a 1 to 1·5-cm incision. As the rats began to awaken from ether anesthesia, [^{14}C]pentobarbital in saline (15 mg/kg; 0·2 ml/300 g; sp. act. 0·70 μCi/mg) was injected into the jugular vein during a 30-sec interval and the incision was sutured. At specific time intervals after the end of the injection, 50-μl samples of tail tip blood were taken for the measurement of unmetabolized [^{14}C]pentobarbital by liquid scintillation counting. The method used[16] includes a differential extraction for separating pentobarbital from its metabolites. The concentration of pentobarbital/ml of blood at each sampling time was converted to the percentage of the total dose injected. Disappearance curves were plotted and linear regression lines of log concentration versus time were calculated by the method of least squares.

Pentobarbital metabolism in vitro. Rats were decapitated and livers removed, weighed and homogenized with 2 vol. of 1·15% KCl in a glass Potter Elvehjem homogenizer using a motor-driven Teflon pestle. The homogenates were centrifuged at 10,000 *g* for 30 min at 5°, and supernatants were decanted and recentrifuged for an additional 20 min. Two-ml samples of the resulting supernatants were used as the enzyme preparations. Protein concentrations in the preparations were determined by the biuret reaction.[17] In addition, the incubation mixtures consisted of NADP, 12·5 μmoles; glucose 6-phosphate, 50 μmoles; nicotinamide, 20 μmoles; $MgCl_2$, 50 μmoles; glucose 6-phosphate dehydrogenase, disodium salt, 1 unit; all of which were added in 2 ml of 0·1 M phosphate buffer, pH 7·4. Pentobarbital sodium, to yield a final concentration of 2×10^{-4} or 1×10^{-3} M, was added in 1 ml of distilled water to start the reaction. Samples were incubated for 20 min in a Dubnoff shaker at 37°. The reaction was stopped by shaking the samples in an ice-water bath and 3 ml of each sample was then assayed for the pentobarbital remaining.[18] In all experiments, samples were run in duplicate.

In three experiments, the 10,000 *g* supernatant was centrifuged at 105,000 *g* for 1 hr and the resulting microsomal pellet was washed by resuspension and reprecipitation. The final pellet was resuspended in 1·15% KCl solution in a volume equal to that of the initial sample of 10,000 *g* supernatant. Incubations with 2-ml portions of the microsomal preparation were carried out under the conditions described above, except that 2 units rather than 1 unit of glucose 6-phosphate dehydrogenase was added.

Kinetics of pentobarbital metabolism in vitro. The kinetics of pentobarbital metabolism in the absence and presence of cannabinoids were studied using the 10,000 *g* supernatants of liver homogenates, prepared as described above, from untreated animals. All cannabinoids were added 5 sec before pentobarbital. THC was added, suspended in 0·2 ml of rat serum, whereas CBN and CBD were added, dissolved in 10 μl of 95% ethanol. This was necessary because CBN and CBD caused precipitation of serum protein while THC did not. Therefore, appropriate control samples contained either 0·2 ml of rat serum or 10 μl of ethanol without cannabinoids. The

incubation mixtures were the same as described above, except that the phosphate buffer volume was reduced to 1·8 ml in each vessel in the THC experiments and to 1·99 ml in the CBN and CBD experiments. Data were plotted according to Lineweaver and Burk[19] and the Michaelis[20] K_m and V_{max} were calculated from linear regression lines which were obtained by the method of least squares. Approximate K_i values for THC, CBN and CBD, using the cannabinoid concentration of 4×10^{-5} M, were calculated by the following formula[21]:

$$\frac{K_m'}{V_{max}'} = \frac{K_m}{V_{max}}\left(1 + \frac{I}{K_i}\right)$$

where K_m' and K_m are the Michaelis constants of the inhibited and unhibited reactions, respectively; V_{max}' and V_{max} are the maximum velocities of the inhibited and uninhibited reactions, respectively; and I is the inhibitor concentration.

Chemicals. Δ^1-THC (99 per cent purity as determined by GLC) was synthesized by Dr. O. Meresz, Department of Chemistry, University of Toronto.[14] CBD (100 per cent) and CBN (98 per cent) were supplied by Makor Chemicals, Jerusalem. NADP, glucose 6-phosphate, glucose 6-phosphate dehydrogenase and nicotinamide were purchased from Sigma Biochemicals. Pentobarbital sodium was obtained from Ingram & Bell, Toronto.

RESULTS

Pentobarbital-induced sleeping time. Treatment of rats with single doses of marijuana extract, lot I, substantially prolonged pentobarbital-induced sleeping time (Table 1). Sleep was prolonged by 100 per cent when marijuana was administered 21·5 hr before the test dose of pentobarbital. The effect gradually decreased to less than 1 per cent as the time interval between cannabis and pentobarbital doses was increased up to 84 hr. Marijuana treatment did not influence the sleep induction time. CBD, at a dose of 23·4 mg/kg (an amount which was equal to that, as measured by GLC, in the dose of marijuana extract, lot I) given 21·5 hr before pentobarbital, increased sleeping time by 130 per cent (Table 2), and had no effect on sleep induction time. An analysis of variance showed that the increases of 100 and 130 per cent were not significantly different ($t = 1{\cdot}20$; df = 38). However, pretreatment with either marijuana extract, lot II, or the equivalent dose of THC (10 mg/kg) did not produce a significant effect.

In experiments with either marijuana extract, lot II, or THC alone, the treated and control groups did not differ with respect to the blood concentrations of pentobarbital at the time of awakening (Table 3). However, animals treated with either marijuana extract, lot I, or CBD alone, 21·5 hr before pentobarbital administration, awoke with higher pentobarbital blood levels than their olive oil controls. When a 40-hr interval was left between doses of marijuana extract, lot I, or olive oil and pentobarbital, the pentobarbital blood levels on awakening were no longer significantly different in the treated and control groups.

Pentobarbital disappearance in vivo. [^{14}C]pentobarbital blood levels decreased more slowly ($P < 0{\cdot}001$) in rats which were treated 21·5 hr earlier with a single dose of marijuana extract, lot I, than in control animals (Fig. 1). The initial concentration of pentobarbital in the blood, indicated by extrapolation of the curve to zero time, was equivalent to 21·7 μg/ml. This is in good agreement with the concentrations

TABLE 1. EFFECT OF A SINGLE DOSE OF MARIJUANA EXTRACT, LOT I (83·32 mg/kg) ON PENTOBARBITAL-INDUCED SLEEP

Time between marijuana extract or olive oil and pentobarbital doses (hr)	Sleep induction time (min ± S.E.M.)		Sleeping time (min ± S.E.M.)		% Increase in sleeping time of treated group
	Control (olive oil)	Treated (extract)	Control (olive oil)	Treated (extract)	
21·5	4·34 ± 0·18(16)*	4·20 ± 0·34(10)	39·0 ± 2·1(16)	78·0 ± 4·8(10)	100†
40	3·73 ± 0·20(14)	3·84 ± 0·22(7)	38·3 ± 4·0(14)	66·2 ± 4·7(7)	73†
63	4·03 ± 0·11(8)	4·30 ± 0·21(11)	32·4 ± 1·7(8)	46·2 ± 3·1(11)	42†
84	3·88 ± 0·16(4)	3·70 ± 0·18(8)	39·6 ± 1·1(4)	39·9 ± 2·3(8)	<1

* Number of rats in parentheses.
† Significant increase $P < 0·005$.

TABLE 2. EFFECT OF CBD, Δ^1-THC AND MARIJUANA EXTRACT, LOT II, ON PENTOBARBITAL-INDUCED SLEEP

Cannabis preparation*	Sleeping time (min ± S. E. M.) Control	Treated	% Increase in sleeping time of treated group
CBD (23·4 mg/kg)	43·7 ± 2·5(8)†	100·6 ± 5·3(8)	130‡
Δ^1-THC (10 mg/kg)	36·5 ± 2·4(7)	36·4 ± 2·0(8)	0
Marijuana extract, lot II (26·2 mg/kg)	43·7 ± 2·5(8)	46·1 ± 3·8(8)	6

* Drug preparations were given 21·5 hr before pentobarbital (30 mg/kg).
† Number of rats in parentheses.
‡ Significant increase $P < 0.001$.

TABLE 3. BLOOD CONCENTRATIONS OF PENTOBARBITAL AT THE TIME OF AWAKENING

Type of pretreatment	Pentobarbital concentration on awakening (μg/ml ± S. E. M.)	P*
Before pentobarbital (21·5 hr):		
Control, olive oil	10·40 ± 0·98(12)†	
Marijuana extract, lot I	16·12 ± 2·84(6)	<0·05
Marijuana extract, lot II	9·70 ± 0·30(8)	>0·10
THC, 10 mg/kg	12·87 ± 2·90(6)	>0·10
CBD, 23·4 mg/kg	16·89 ± 2·29(8)	<0·05
Before pentobarbital (40 hr):		
Control, olive oil	11·40 ± 2·46(6)†	
Marijuana extract, lot I	14·63 ± 2·41(8)	>0·10

* Comparison made with control at appropriate time (21·5 or 40 hr).
† The 21·5- and 40-hr control values not significantly different; number of rats in parentheses.

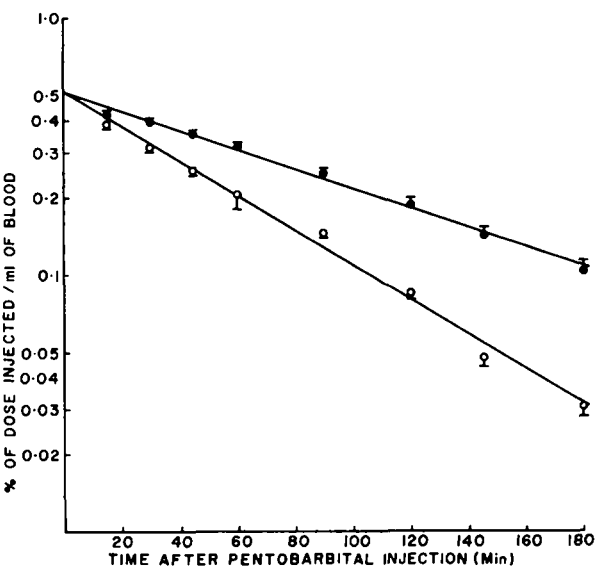

FIG. 1. Disappearance of [^{14}C]pentobarbital from blood of rats injected intravenously at 21·5 hr after pretreatment with marijuana extract, lot I, or olive oil control. Marijuana-pretreated rats, ● (n = 10); controls, ○ (n = 9). Vertical bars represent S. E. M. The zero time concentration of pentobarbital corresponds to 21·7 μg/ml of blood.

found in earlier work[15] during light pentobarbital anesthesia. The $T_{1/2}$ for the disappearance of pentobarbital in the treated group was 80 min compared to 45 min in the control group, representing a 44 per cent decrease in the rate of pentobarbital metabolism in the treated animals. The apparent volumes of distribution of pentobarbital in the two groups were the same since the extrapolated disappearance curves have identical intercepts.

Pentobarbital metabolism in vitro. Preliminary experiments were carried out to determine optimal conditions for the metabolism of pentrobarbital by liver preparations *in vitro*. Metabolism was found to be linear with increasing amounts of 10,000 *g* supernatant up to 3 ml (from 1·5 g liver), and with increasing time up to 30 min. The cofactor concentrations used were determined to be appropriate for both pentobarbital and Δ^1-THC metabolism.

When the initial substrate concentration was 2×10^{-4} M, inhibition of pentobarbital metabolism gradually decreased from 53 per cent at 21·5 hr after a single dose of marijuana extract, lot I, to 15 per cent (not significant) after an 84-hr interval (Table 4). In a separate series of three experiments at 21·5 hr after treatment, the inhibition of pentobarbital metabolism was 45 per cent when the initial substrate concentration was 2×10^{-4} M and 41 per cent at 1×10^{-3} M. However, this effect could not be demonstrated with either marijuana extract, lot II, or with THC (10 mg/kg). CBD, at doses of 23·4 mg/kg and 0·88 mg/kg (amounts equal to those assayed by GLC in the doses of marijuana extract, lots I and II, respectively), inhibited metabolism by 41 and 0 per cent, respectively, when administered 21·5 hr before animal sacrifice. Addition of CBD to marijuana extract, lot II, to provide a final dose of 23·4 mg/kg resulted in 45 per cent inhibition of pentobarbital metabolism (Table 4). The control rates of pentobarbital metabolism varies with different groups of rats received from the supplier.

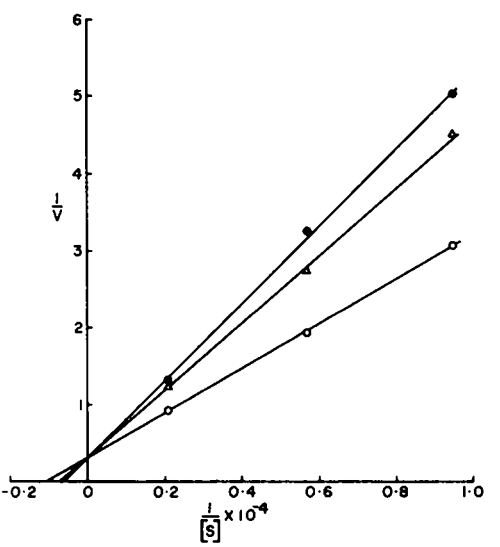

FIG. 2. Inhibition of pentobarbital metabolism *in vitro* by CBD. [S] = molar concentration of pentobarbital. V = μmoles pentobarbital metabolized/g liver/20 min. Control, ○; CBD, 4×10^{-5} M, △; CBD, 2×10^{-4} M, ●.

TABLE 4. EFFECT OF CANNABIS PREPARATIONS ON PENTOBARBITAL METABOLISM BY RAT LIVER *in vitro*

Cannabis preparation and dose	Time between administration and sacrifice (hr)	% Pentobarbital metabolized*		% Inhibition of metabolism in treated group	P
		Control	Treated		
Marijuana extract, lot I, 83·3 mg/kg†	21·5	39·87 ± 4·61(10)‡	18·68 ± 2·63(9)	53	<0·005
	40	52·77 ± 1·86(7)	30·57 ± 1·46(11)	42	<0·001
	63	44·81 ± 2·39(5)	35·59 ± 1·71(7)	21	<0·01
	84	35·14 ± 1·73(6)	29·74 ± 3·16(6)	15	>0·10
Marijuana extract, lot II, 26·2 mg/kg	21·5	32·14 ± 2·23(9)	31·63 ± 2·28(9)	2	N.S.§
Δ^1-THC, 10 mg/kg	21·5	48·93 ± 1·06(6)	48·12 ± 2·29(8)	2	N.S.
CBD, 23·4 mg/kg	21·5	30·46 ± 2·27(7)	18·06 ± 2·04(5)	41	<0·005
CBD, 0·88 mg/kg	21·5	30·46 ± 2·27(7)	30·51 ± 3·31(4)	0	N.S.
Marijuana extract, lot II, 26·2 mg/kg, plus CBD, 22·52 mg/kg	21·5	27·68 ± 1·36(9)	15·25 ± 1·95(9)	45	<0·005

* Initial pentobarbital concentration was 2×10^{-4} M; enzyme preparation = 10,000 *g* supernatant.

† Composition of the two marijuana extracts is given in the text.

‡ Values shown are mean ± S.E.M.; number of rats given in parentheses.

§ N.S. = not significant.

TABLE 5. EFFECT OF CANNABINOIDS ON KINETICS OF PENTOBARBITAL METABOLISM *in vitro*

Cannabinoid (M)	K_m ($\times 10^{-4}$ M)	V_{max}*	K_i ($\times 10^{-5}$ M)
Serum control	6·59 ± 0·44(5)†	1·65 ± 0·12	
THC (4×10^{-5})	8·88 ± 0·90(6)‡	1·99 ± 0·29	28·9
THC (1×10^{-3})	11·58 ± 1·63(4)‡	2·19 ± 0·29	
Ethanol control	10·35 ± 0·82(11)	3·30 ± 0·18	
CBN (4×10^{-5})	11·70 ± 0·58(4)‡	3·45 ± 0·07	23·2
CBN (1×10^{-4})	14·06 ± 0·86(4)‡	3·82 ± 0·13	
CBD (4×10^{-5})	16·89 ± 2·30(6)‡	3·73 ± 0·41	9·26
CBD (2×10^{-4})	20·78 ± 4·42(6)‡	3·83 ± 0·56	

* V_{max} = μmoles pentobarbital metabolized/g liver/20 min.
† Values shown are mean ± S.E.M.; number of rats given in parentheses.
‡ Values significantly ($P < 0·05$) different from appropriate control values.

Pretreatment of rats with marijuana extract, lot I, 21·5 hr before sacrifice resulted in an inhibition of pentobarbital metabolism even when a microsomal preparation rather than the 10,000 *g* supernatant of rat liver was used. Microsomal pentobarbital metabolism was inhibited by 56 per cent at an initial pentobarbital concentration of 2×10^{-4} M and by 39 per cent at 1×10^{-3} M.

Kinetics of pentobarbital metabolism in vitro. A Lineweaver and Burk plot of the inhibition of pentobarbital metabolism *in vitro* by CBD is shown in Fig. 2. Similar but less marked results were obtained with THC and CBN. The inhibition of pentobarbital metabolism caused by THC, CBN and CBD appeared to be competitive since the V_{max} values of metabolism (Table 5) in the presence of cannabinoids were the same in all cases as those of the appropriate controls, while the K_m increased significantly with all but the lowest concentration of CBN.

The control K_m and V_{max} values for pentobarbital metabolism in the presence of ethanol (32·6 mM) were significantly greater than in the presence of serum. Therefore, the kinetic parameters for THC were compared with the serum control values, and the values for CBN and CBD were compared with the ethanol control values. THC, at both 4×10^{-5} M and 1×10^{-3} M, caused a significant increase in the K_m as did CBD at both 4×10^{-5} M and 2×10^{-4} M. Although CBN at 1×10^{-4} M caused a significant increase in the K_m, the increase at 4×10^{-5} M was not significant. The K_i values, calculated at 4×10^{-5} M for all cannabinoids, indicate that the metabolic inhibitory potency of CBD *in vitro* is three times that of THC, while THC is about twice as potent an inhibitor as CBN.

DISCUSSION

This study clearly shows that the prolongation of pentobarbital-induced sleeping time in rats after an acute dose of marijuana extract, lot I, was paralleled for at least 63 hr by a decreased rate of pentobarbital metabolism *in vitro*. Eight-four hr after the marijuana dose, sleep was not prolonged and pentobarbital metabolism *in vitro* was not inhibited significantly. The 44 per cent decrease in rate of pentobarbital disappearance from the blood of rats which had been treated with marijuana extract, lot I, 21·5 hr before pentobarbital administration, can almost exactly account for the 100 per cent increase in sleeping time.

In this study, pretreatment of rats with marijuana extract, lot II, did nor affect pentobarbital sleeping time or pentobarbital metabolism *in vitro* in rat liver even though the THC dose given in both extracts was 10 mg/kg. In addition, treatment of rats with pure THC (10 mg/kg) also failed to have an effect. Therefore, THC, at the dose used, could not account for the long-lasting effect of marijuana extract, lot I.

On the other hand, cannabis resin (500 mg/kg), containing 55·1% THC, has recently been reported[22] to induce benzpyrene hydroxylase activity in rat liver and lung when measured at 24 and 48 hr after a single dose of resin. However, this effect was not reproduced in rat liver but was demonstrated in part in rat lung when Δ^1-THC (280 mg/kg) was given 24 hr before assay. The apparent contradiction between the results of the cited study and the current investigation may be due to the vastly different contents of THC in the marijuana preparations used, resulting in THC doses of 280 mg/kg and 10 mg/kg respectively. In addition, it is not known how much of the THC content in the cannabis resin was present in the form of THC acid. Perhaps a high content of cannabinoids in their acid forms or non-cannabinoid components of cannabis could produce induction of metabolism. The content of CBN and CBD in the cannabis resin was not reported.

Subsequently, Marcotte and Witschi,[23] using cannabis from the same lot as our marijuana, lot II, found that benzpyrene hydroxylase activity could be stimulated in rat lung and liver for up to 24 hr by exposure of animals to cannabis smoke, even when the cannabinoids had been previously extracted. This would presumably indicate that the effect was due to cyclic hydrocarbons produced by combustion, some of which are known to be potent inducers of drug metabolism.[24]

THC has been shown[7,8] to prolong pentobarbital and barbital sleeping time when the barbiturates were given within 40 min of the THC. Under these conditions the THC concentration in the liver could possibly become high enough to interfere with pentobarbital metabolism. We and others[10,25,26] have found that THC does inhibit drug metabolism when added *in vitro*. However, this would not account for the effect seen with barbital, since barbital is not metabolized. The possibility of synergism in the central nervous system must, therefore, be considered.[7]

Our studies indicate that pure CBD, when given at a dose of 23·4 mg/kg, which is the amount also given in marijuana extract, lot I, can account almost entirely for the cannabis-induced effects seen on pentobarbital sleep and pentobarbital metabolism. Equivalent doses of marijuana extract, lot I, and of pure CBD produced increases in sleeping time of 100 and 130 per cent, respectively, and inhibited pentobarbital metabolism *in vitro* by 53 and 41 per cent respectively. Although the molecular weight of the microsomal enzyme(s) involved in pentobarbital metabolism is uncertain, a conservative estimate suggests that the molar ratio of enzyme to K_m for pentobarbital is 0·5 or less. This would mean that the system *in vitro* was operating at the boundary between zones A and B as defined by Straus and Goldstein.[27] The fact that the prolongation of $T_{1/2}$ for pentobarbital *in vivo* and the inhibition of metabolism *in vitro* agreed closely suggests that the use of a relatively low substrate concentration *in vitro* is appropriate.

The finding that pentobarbital metabolism *in vitro* was dramatically inhibited after marijuana extract, lot I, treatment, even when washed microsomes rather than 10,000 *g* supernatants were used, suggests that CBD remained associated with microsomal membranes by virtue of its high lipid solubility or perhaps some form of chemical

bonding. Recently Dingell *et al.*[28] have demonstrated that THC remains associated with rat liver nuclear and microsomal membranes during their preparation. Alternatively, CBD may have interfered with microsomal enzyme turnover.

Analysis of variance showed that there was no significant difference between the effects produced by marijuana extract, lot I, and CBD on either sleeping time or pentobarbital metabolism. In addition, these studies show that CBN and THC can competitively inhibit pentobarbital metabolism when added *in vitro*, but much less effectively than CBD.

It is interesting that the blood concentration of pentobarbital at the time of awakening was significantly higher in rats treated with either marijuana extract, lot I, or CBD (23·4 mg/kg) 21·5 hr before pentobarbital injection than in control animals. This can not be explained simply by inhibition of pentobarbital metabolism. One possibility is that CBD has, in addition, an effect on the brain which is antagonistic to that of pentobarbital. A more probable explanation is that prolongation of the exposure to pentobarbital permits a greater development of acute tolerance to it.[29,30]

Marijuana extract, lot II, and pure CBD at an equivalent low dose (0·88 mg/kg) had no influence on pentobarbital sleep or pentobarbital metabolism *in vitro*. Addition of CBD to marijuana extract, lot II, to produce a CBD dose of 23·4 mg/kg (equivalent to marijuana extract, lot I) resulted in the inhibition of metabolism *in vitro*. The inhibitory effect of CBD on pentobarbital metabolism is, therefore, dose dependent. The magnitude of effects at the two doses are compatible with the three-point dose–response curve for CBD and the four-point dose–response curve for cannabis extract shown by Paton and Pertwee,[10] when allowance is made for the fact that they gave the barbiturate at 30 min rather than 21·5 hr after the cannabis preparations. THC is known to be absorbed satisfactorily after oral administration to animals and man,[31–33] and there is no reason to doubt that other cannabinoids would also be absorbed. Therefore, though the ratio of CBD doses used here cannot be assumed to equal the ratio of resulting blood levels of CBD, a dose-related effect is unquestionable.

The prolongation of pentobarbital sleeping time in the rat after a dose of marijuana extract appears primarily due to the presence of CBD which inhibits pentobarbital metabolism. It is important that, in studies utilizing cannabis extracts, the content of THC, CBD and other cannabinoids be known, since the effects observed may depend on any one or all of the cannabinoids present.

Acknowledgements The authors wish to express appreciation to Miss Grace Lee for excellent technical assistance and to Dr. L. Endrenyi for statistical analysis of the data.

REFERENCES

1. R. C. Pillard, *New Engl. J. Med.* **283,** 294 (1970).
2. W. McGlothlin, K. Jamison and S. Rosenblatt, *Nature, Lond.* **228,** 1227 (1970).
3. L. E. Hollister, *Science, N.Y.* **172,** 21 (1971).
4. A. S. Carlin and R. D. Post, *J. Am. med. Ass.* **218,** 867 (1971).
5. B. C. Bose, A. Q. Saifi and A. W. Bhagwat, *Archs. int. Pharmacodyn. Ther.* **141,** 520 (1963).
6. S. Garattini, in *Hashish: Its Chemistry and Pharmacology* (Eds. G. E. W. Wolstenholme and J. Knight), p. 70. Little, Brown, Boston (1965).
7. R. K. Kubena and H. Barry, III, *J. Pharmac. exp. Ther.* **173,** 94 (1970).
8. R. D. Sofia and H. Barry, III, *Eur. J. Pharmac.* **13,** 134 (1970).
9. J. C. Garriott, L. J. King, R. B. Forney and F. W. Hughes, *Life Sci.*, Pt. I, **6,** 2119 (1967).
10. W. D. M. Paton and R. G. Pertwee, *Br. J. Pharmac. Chemother.* **44,** 250 (1972).

11. G. JONES and R. G. PERTWEE, *Br. J. Pharmac. Chemother.* **45,** 375 (1972).
12. W. D. M. PATON and D. M. TEMPLE, *Br. J. Pharmac. Chemother.* **44,** 346P (1972).
13. S. AGURELL, I. M. NILSSON, A. OHLSSON and F. SANDBERG, *Biochem. Pharmac.* **19,** 1333 (1970).
14. M. D. WILLINSKY, Ph.D. Thesis, University of Toronto (1971).
15. H. KALANT, J. M. KHANNA and J. MARSHMAN, *J. Pharmac. exp. Ther.* **175,** 318 (1970).
16. E. RUBIN, H. GANG, P. S. MISRA and C. S. LIEBER, *Am. J. Med.* **49,** 801 (1970).
17. A. G. GORNALL, C. J. BARDAWILL and M. M. DAVID, *J. biol. Chem.* **177,** 751 (1949).
18. B. B. BRODIE, J. J. BURNS, L. C. MARK, P. A. LIEF, E. BERNSTEIN and E. M. PAPPER, *J. Pharmac. exp. Ther.* **109,** 26 (1953).
19. H. LINEWEAVER and D. BURK, *J. Am. chem. Soc.* **56,** 658 (1934).
20. L. MICHAELIS and M. L. MENTEN, *Biochem. Z.* **49,** 333 (1913).
21. M. DIXON and E. C. WEBB, *Enzymes* (2nd Ed.), p. 327. Academic Press, New York (1964).
22. H. P. WITSCHI and B. SAINT-FRANCOIS, *Toxic. appl. Pharmac.* **23,** 165 (1972).
23. J. MARCOTTE and H. P. WITSCHI, *Res. Commun. Chem. Path. Pharmac.* **4,** 561 (1972).
24. A. H. CONNEY, *Pharmac. Rev.* **19,** 317 (1967).
25. J. V. DINGELL, H. G. WILCOX and H. A. KLAUSNER, *Pharmacologist* **13,** 296 (1971).
26. G. M. COHEN, D. W. PETERSON and G. J. MANNERING, *Life Sci.*, Pt. 1, **10,** 1207 (1971).
27. O. H. STRAUS and A. GOLDSTEIN, *J. gen. Physiol.* **26,** 559 (1943).
28. J. V. DINGELL, K. W. MILLER, E. C. HEATH and H. A. KLAUSNER, *Biochem. Pharmac.* **22,** 949 (1973).
29. E. W. MAYNERT and G. I. KLINGMAN, *J. Pharmac. exp. Ther.* **128,** 192 (1960).
30. H. KALANT, A. E. LEBLANC and R. J. GIBBINS, *Pharmac. Rev.* **23,** 135 (1971).
31. R. J. FREUDENTHAL, J. MARTIN and M. E. WALL, *Br. J. Pharmac. Chemother.* **44,** 244 (1972).
32. R. B. FORNEY, *Pharmac. Rev.* **23,** 279 (1971).
33. L. LEMBERGER, J. AXELROD and I. J. KOPIN, *Pharmac. Rev.* **23,** 371 (1971).