Metabolism of Δ^1-Tetrahydrocannabinol by Rats Tolerant to Cannabis[1]

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Daily treatment of rats with phenobarbital, 75 mg/kg, for 5 days resulted in an induction of the metabolism of ^{14}C-Δ^1-*trans*tetrahydrocannabinol (THC) *in vitro* and *in vivo*. Although the K_m and V_{max} for THC metabolism by liver preparations *in vitro* were not altered, the rate of formation of highly polar THC metabolites was significantly increased and the final concentration of 7-hydroxy-Δ^1-THC was decreased. Phenobarbital also significantly increased the rate of biliary excretion of THC and its metabolites and their disappearance from the blood and tissues.

In one experiment, rats receiving a marihuana extract daily (providing THC, 5–10 mg/kg) failed to gain body weight during the first week of treatment, but then became tolerant to this effect. But neither the rate of THC metabolism *in vitro* nor the pattern of metabolites formed was influenced in the tolerant animals compared to controls. In another experiment, rats were shown to become tolerant on chronic treatment with marihuana extract, as indicated by a reduction in marihuana-induced enhancement of ethanol sleeping time. The biliary excretion, disappearance from the blood, and residual tissue levels of THC and its metabolites were not altered in the tolerant animals compared to pair-fed and *ad libitum*-fed controls. Therefore the mechanism of tolerance development to cannabis in the rat is more likely of CNS than of metabolic origin.

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L'administration de phénobarbital (75 mg/kg par jour durant 5 jours) produit chez le rat une accélération *in vivo* et *in vitro* du métabolisme hépatique du ^{14}C-Δ^1-tétrahydrocannabinol (THC). Bien que le K_m et la V_{max} du métabolisme du THC par des préparations microsomales de foie ne soient pas modifiés, nous observons une augmentation de la vitesse de formation de métabolites hautement polaires du THC et une diminution de la concentration finale du 7-hydroxy-Δ^1-THC. Le phénobarbital produit également une accélération de l'excrétion biliaire des métabolites du THC et de sa disparition du sang et des tissus. Des rats ayant reçu quotidiennement un extrait de marihuana (correspondant à 5–10 mg/kg) n'ont pas présenté d'augmentation de poids corporel après 1 semaine mais ont présenté par la suite une tolérance aux effets du THC. Comparativement aux animaux témoins, les rats tolérants ne démontrent aucune modification de la vitesse du métabolisme *in vitro* du THC ni du type de métabolites formés. Dans une autre série d'expériences, la tolérance aux effets de la marihuana a été démontrée par la réduction du prolongement induit par la marihuana du sommeil à l'éthanol. L'excrétion biliaire, la disparition du sang et la teneur résiduelle des tissus en THC et métabolites ne sont pas modifiées chez l'animal tolérant, comparativement à l'animal témoin soumis à un même régime alimentaire ou à des animaux témoins nourris *ad libitum*. Nous concluons que la tolérance au THC resulte de l'adaptation du système nerveux central plutôt que d'une adaptation métabolique. [Traduit par le journal]

Introduction

Tolerance to the effects of cannabis and its primary psychoactive constituent, Δ^1-tetrahydrocannabinol (THC), develops in animals and man (McMillan *et al.* 1971; C.I.N.M.U.D. 1972; Paton and Pertwee 1973) following chronic administration of cannabis preparations. However, the mechanisms of this tolerance development have not been clearly established. Lemberger *et al.* (1971) have suggested that chronic marihuana smoking by humans may result in an increase in the rate of THC metabolism. However, their findings are not

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necessarily conclusive, since all five chronic smokers in the study were males and three of the four non-users were female (Lemberger *et al.* 1970, 1971) (Lemberger, L.: personal communication). Sex differences in cannabinoid metabolism have not been ruled out. Furthermore, although the subjects did not use "other drugs or medications," their use of alcohol and tobacco is not discussed. Ethanol (Kater *et al.* 1969; Rubin and Lieber 1968), as well as cyclic hydrocarbons in tobacco smoke (Conney 1967), have been reported to act as inducers of microsomal drug metabolism.

The present studies were carried out to determine to what extent, if any, induction of THC metabolism might contribute to cannabis tolerance in the rat. For this purpose, tolerance to cannabis was assessed by the reduction of its enhancement of the depressant action of ethanol (Kalant and LeBlanc 1974).

Materials and Methods

Materials

^{14}C-(—)-3,4-*trans*-Δ^1-tetrahydrocannabinol (THC) and non-labelled THC were synthesized as described by Willinsky *et al.* (1974). The monoterpenoid nomenclature is employed in this paper; in the dibenzopyran nomenclature this compound is designated Δ^9-THC. The chemical purity of both compounds following preparative thin-layer chromatography (TLC) was >98% as determined by gas–liquid chromatography (GLC). Pure 7-hydroxy-Δ^6-tetrahydrocannabinol was provided by Health and Welfare Canada (HWC), Ottawa, through the courtesy of Mr. R. A. Graham. Nicotinamide, NADP, glucose 6-phosphate disodium salt, and glucose 6-phosphate dehydrogenase were purchased from Sigma Chemicals. TLC was carried out with 0.25-mm silica gel G on 20 × 5 cm plastic sheets (Polygram Sil G, Brinkmann Instruments).

Treatment of Rats with Phenobarbital

Male Wistar rats, 260–270 g, were treated for 5 days with one daily intraperitoneal (i.p.) injection of either 0.9% NaCl, 5 ml/kg, or phenobarbital sodium, 75 mg/kg, dissolved in the same volume of 0.9% NaCl. Purina chow and water were available *ad libitum*. THC metabolism *in vitro* or *in vivo* was measured 24 h after the last dose.

Marihuana Extracts and Animal Treatment

Two lots of marihuana, which differed in their cannabinoid content, were obtained from HWC. Alcoholic extracts were prepared as described previously (Siemens *et al.* 1974). For *in vitro* metabolism studies, male Wistar rats (250 g) were treated by gavage with a marihuana extract (M.E.-I) dissolved in olive oil at a dose that provided 5 mg THC per kilogram for 10 days, followed by 7.5 mg/kg for the next 10 days, and then 10 mg/kg for 8 days. The cannabinoid composition of the olive-oil solution as determined by GLC was: THC, 2.5 mg/ml; cannabidiol (CBD), 5.85 mg/ml; cannabinol (CBN), 1.49 mg/ml. Controls received equal volumes of olive oil by gavage. Food and water were available *ad libitum* during the first 15 days of treatment, but thereafter food intake of the controls was restricted to the amount voluntarily consumed by the cannabis group (15–18 g per day) to eliminate the possibility that different amounts of diet would interfere with a drug effect. The rats were killed at 21.5, 40, 63, or 84 h after the last dose of M.E.-I or olive oil.

For biliary excretion studies, rats were intubated daily for 13–14 days with another marihuana extract (M.E.-II) dissolved in olive oil at a dose that also provided THC, 10 mg/kg. The cannabinoid composition of this olive-oil solution was: THC, 2.5 mg/ml; CBD, 0.22 mg/ml; CBN, 0.46 mg/ml. In all experiments, control animals received olive oil. The biliary excretion of ^{14}C-THC and its metabolites was studied 24–26 h following the last dose of the chronic treatment. In another set of experiments, the biliary excretion of ^{14}C-THC was examined 48 and 72 h following the end of a 15-day treatment with M.E.-II or olive oil.

Measurement of Tolerance to Marihuana

The rats to be used later in biliary excretion studies of ^{14}C-THC were divided into three primary groups of 16 each. Each group was divided into four equal subgroups, each subgroup being scheduled to receive one of four different test doses of ethanol (2.4, 2.7, 3.0, or 3.3 mg/kg i.p. in a 25% (v/v) saline solution). On day 1, two rats of each subgroup received olive oil followed 1.5 h later by ethanol, and the other two rats received a dose of M.E.-II providing THC, 10 mg/kg, followed by ethanol. The duration of ethanol-induced loss of righting reflex was determined (Khanna and Kalant 1970). On day 2, the rats were retested; those that had previously received olive oil were now given M.E.-II, and *vice versa*. In this way the effect of M.E.-II on the dose–response curve for ethanol sleeping time, with each animal as its own control, was determined before the start of chronic treatment with cannabis extract.

During the following 10 days, group I received M.E.-II daily and groups II and III received equivalent volumes of olive oil. Groups I and II were pair-fed, group II rats receiving the amount of Purina chow eaten the day before by the group-I member of each pair. Group III had chow available *ad libitum*. All groups had tap water available *ad libitum* throughout the experimental period.

On days 13 and 14, ethanol sleeping time was again determined in all three groups with the same doses of alcohol and of M.E.-II or olive oil as before. The order of testing with the various combinations was the reverse of that used on days 1 and 2. Upon awakening, rats of group I that had received olive oil as a test dose received the usual dose of M.E.-II to maintain the chronic treatment. Rats in groups II and III that had received olive oil as test dose, as well as those of all three groups that had received M.E.-II as test dose, received olive oil upon awakening.

Animals that were to be used for the determination of biliary excretion of ^{14}C-THC were appropriately treated with olive oil or M.E.-I for one additional day after the final determination of ethanol sleeping time.

Metabolism of THC in vitro

THC complexed with rat serum was incubated with the 10 000 × *g* supernatant (10 KS) of rat-liver homogenates under previously determined optimal conditions (Siemens and Kalant 1974). Initial concentrations of THC ranged from 25 to 143 μM in different experiments. Following incubation for 5, 10, 15, or 30 min, the samples were extracted with methanol and assayed for unchanged THC and its metabolites by TLC and radiochromatographic scanning (Nuclear-Chicago, Actigraph III, radiochromatograph scanner), as described previously (Siemens and Kalant 1974).

Metabolism of THC in vivo

The biliary excretion of ^{14}C-THC and its metabolites was studied in rats treated chronically with phenobarbital and in their controls, as well as in the M.E.-II tolerant animals and their non-tolerant controls. Two or three animals from each alcohol-dose subgroup of each major-treatment group were used in the latter study. The disappearance of THC from the blood during the period of bile collection, as well as the tissue distribution of THC and its metabolites at the end of the experiment, were determined in the same animals. All the techniques have been described in detail elsewhere (Siemens and Kalant 1974).

Results

Induction of THC Metabolism by Phenobarbital

In vitro Studies

Neither the apparent K_m nor the V_{max} (Lineweaver and Burk 1934) nor specific activity of THC metabolism were changed significantly by phenobarbital treatment (Fig. 1), as determined by incubating increasing amounts of THC with 10 KS liver preparations for 10 min. The apparent K_m values were 429 ± 135 μM and 466 ± 116 μM for control and phenobarbital-treated animals, respectively, and the corresponding apparent V_{max} values were 0.49 ± 0.12 and 0.54 ± 0.13 μg THC metabolized per milligram protein per minute.

In keeping with many previous observations in the literature, however, the liver weight relative to the body weight was 42% greater ($P < 0.001$) for phenobarbital-treated rats than for control animals. In addition, the 10 KS protein content (milligrams per gram of liver) was increased by 14% ($P < 0.001$) in phenobarbital-treated animals. Therefore the total capacity of rat liver to metabolize THC was substantially (62%) increased by phenobarbital treatment.

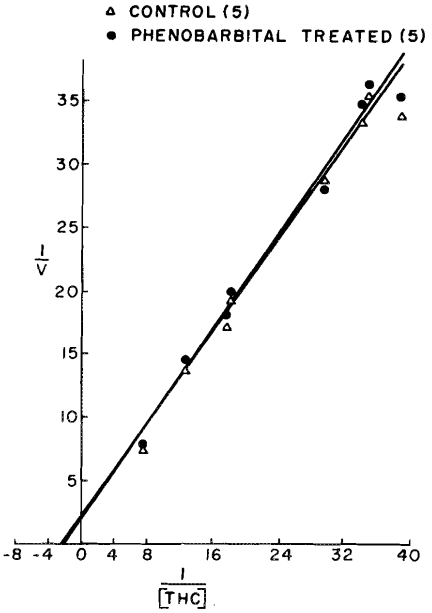

FIG. 1. Effect of chronic phenobarbital treatment of rats on THC metabolism by liver preparations *in vitro*. Points represent mean values of two to four duplicate samples. V, μg THC metabolized per milligram of 10 KS protein per minute; [THC], initial mM concentration of THC.

The rate of production of highly polar THC metabolites was significantly increased by the phenobarbital treatment (Fig. 2). Although the same amount of THC (zone 8) remained at the end of incubation for the two groups, the amount of zone 6, tentatively identified by TLC as 7-hydroxy-Δ^1-THC (7-OH-THC) on the basis of results published by Ho *et al.* (1972), was significantly less for the phenobarbital-treated than the control animals. In contrast, the amounts of radioactivity present in zones 1, 2, 3, 5, and in the non-extractable fraction were significantly greater for treated than control rats, except for zone 3 at the lower initial concentration of THC. These zones represent THC metabolites more polar than 7-OH-THC. Zone 3 was tentatively identified as 6,7-dihydroxy-Δ^1-THC (6,7-diOH-THC) by TLC (Ho *et al.* 1972).

Biliary-Excretion Studies

The rate of biliary excretion of total ^{14}C was significantly greater ($P < 0.05$) in pheno-

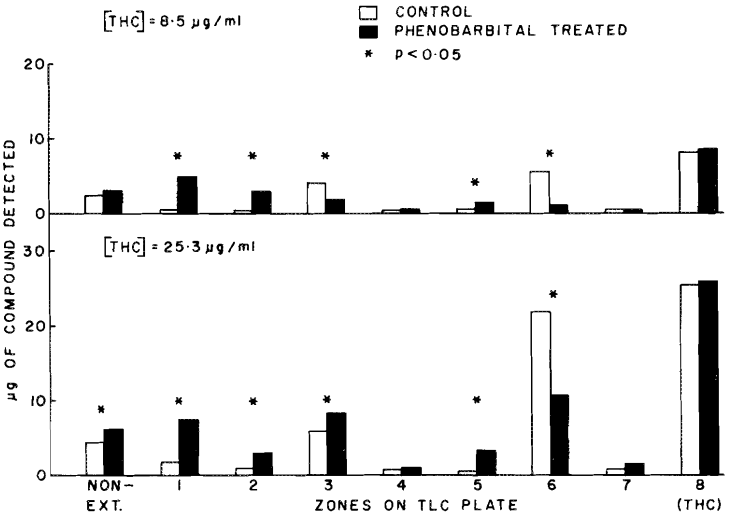

FIG. 2. Effect of chronic phenobarbital treatment of rats on the production of THC metabolites *in vitro* at two different initial THC concentrations. R_f values of zones detected on TLC plates: (1) 0–0.05, (2) 0.13–0.23, (3) 0.29–0.36 (presumed 6,7-diOH-THC), (4) 0.39–0.48, (5) 0.51–0.56, (6) (7-OH-THC) 0.57–0.67, (7) 0.73–0.80, (8) (THC) 0.87–0.96; NON-EXT., fraction that could not be extracted from incubation sample with methanol. N = five animals per treatment group, with duplicate incubations for each preparation at each THC concentration.

barbital-treated rats than in controls during the first 40 min after injection of ^{14}C-THC (Fig. 3). Although total bile flow during the 3-h experimental period was significantly increased by the phenobarbital treatment (3.68 g, controls; 4.67 g, treated; $P < 0.02$), it did not account for the increased rate of ^{14}C excretion. That is, during the first 20 min (the period of greatest difference in ^{14}C excretion between the two groups), the biliary excretion of ^{14}C in the phenobarbital group exceeded that of the controls even when expressed per 100 mg of bile. After 20 min, the rate of biliary excretion of ^{14}C fell more rapidly in the phenobarbital-treated group than in the controls, as the residual level in the blood fell (see below).

The THC-metabolite pattern in the 10–20 min bile samples was found to be the same for the treated and control rats. No unchanged THC was detected, and 7-OH-THC accounted for only about 2% of the total radioactivity. In contrast, 12% was detected at R_f 0.23–0.60, 14% at 0.14–0.22, 55% at 0–0.13, and 17% was non-extractable.

The rate of disappearance of total ^{14}C from the blood was significantly increased ($P < 0.005$) in the phenobarbital-treated rats (Fig. 4). Furthermore, the residual amounts of ^{14}C in the liver and kidneys were significantly decreased in the treated animals (Table 1). The concentration in the lung would also have been

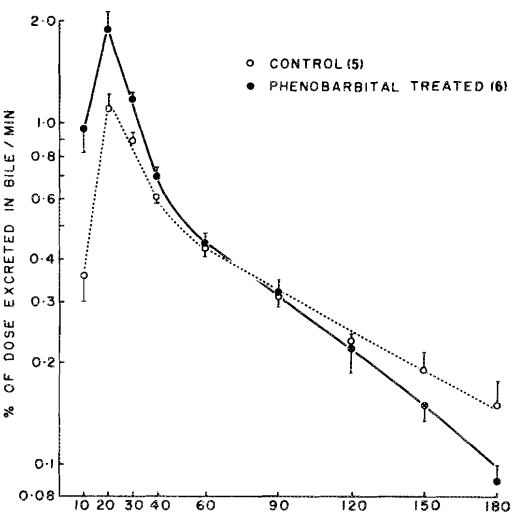

FIG. 3. Effect of chronic treatment of rats with phenobarbital on the rate of biliary excretion of total ^{14}C activity after i.v. injection of ^{14}C-THC. Values represent means, and bars indicate S.E.M., with numbers of animals in parentheses.

TABLE 1. Effect of chronic phenobarbital and M.E.-II treatments on tissue distribution of total radioactivity after i.v. injection of ^{14}C-THC

Treatment	Percentage of ^{14}C dose present in tissues and urine 3 h after injection				
	Liver	Lungs	Brain	Kidneys	Urine
Phenobarbital Na (75 mg/kg per day; 5 days; $N = 6$)	2.95 ± 0.15**	0.05 ± 0.02	—	0.15 ± 0.04*	0.97 ± 0.15
Saline control (5 days; $N = 5$)	4.94 ± 0.29	0.09 ± 0.02	—	0.28 ± 0.01	0.98 ± 0.14
M.E.-II in olive oil (THC, 10 mg/kg per day; 15 days; $N = 9$)	4.45 ± 0.22	0.10 ± 0.01	0.07 ± 0.02	0.31 ± 0.03	0.71 ± 0.16
Pair-fed control (15 days; $N = 7$)	4.04 ± 0.27	0.11 ± 0.03	0.06 ± 0.02	0.31 ± 0.03	0.69 ± 0.16
Ad libitum-fed control (15 days; $N = 8$)	4.02 ± 0.31	0.07 ± 0.02	0.07 ± 0.01	0.26 ± 0.03	0.58 ± 0.08

NOTE: Values shown are means ± S.E. Significance of difference from control: *$P < 0.02$; **$P < 0.01$.

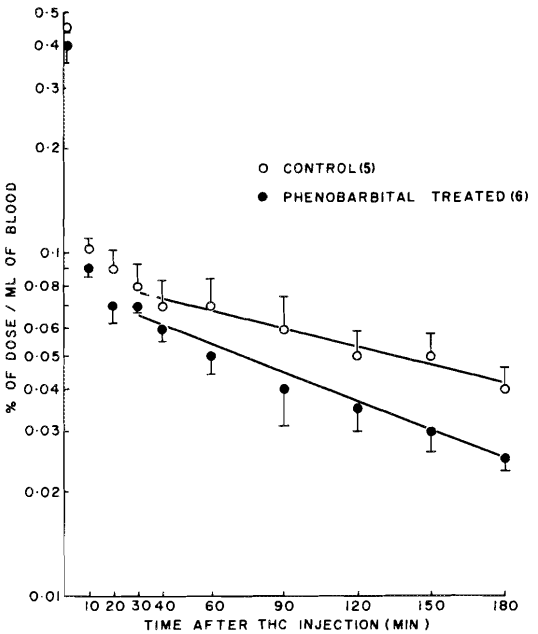

FIG. 4. Effect of chronic treatment of rats with phenobarbital on the rate of ^{14}C disappearance from the blood following ^{14}C-THC injection. Values represent means ± S.E.M. Linear regressions were calculated by the method of least squares with points from 30 to 180 min.

significantly reduced but for a single aberrantly high value (0.11% compared with 0.03–0.05% in the other animals). The ^{14}C concentration in the urine was not altered.

Tolerance of Rats to Marihuana

Tolerance to M.E.-I

Fig. 5 shows the effect of chronic treatment with M.E.-I on the change in body weight. Control rats increased in weight constantly, whereas the treated animals failed to gain for the first 6–7 days of treatment, probably because of reduced food and water intake (Manning *et al.* 1971, Järbe and Henrikson 1973). Thereafter they gained as rapidly as the controls. Increasing the dose of M.E.-I at days 11 and 21 did not interfere with the weight gain. After the 15th day, the rate of weight gain in both groups of rats was slightly reduced because food was restricted. These findings demonstrate that the treated rats developed tolerance to an initial adverse effect of marihuana extract.

Tolerance to M.E.-II

Analyses of variance demonstrated that the ethanol-induced sleeping time for all 3 groups of animals was not dependent on the order of treatment on test days 1 and 2 or 13 and 14 (Table 2). Therefore the data from the two orders of treatment were combined for final analyses.

The ethanol-induced sleeping time was clearly longer following a single test dose of M.E.-II than after olive oil in all three groups of rats before the chronic-treatment period (Fig. 6, Table 3). Following the 10-day treatment period, the ethanol-induced sleeping time was again markedly increased by an acute dose of M.E.-II in both control groups, but the effect was significantly decreased in the chronic M.E.-II treated animals compared with the pair-fed and *ad libitum* controls (Fig. 6, Table 3, 4). At an ethanol dose of 2.4 g/kg there appeared to be complete tolerance to M.E.-II, *i.e.* the animals treated chronically with M.E.-II showed no enhancement of sleeping with either olive oil or M.E.-II. At the three highest doses of ethanol, M.E.-II still produced an apparent slight increase in the sleeping time (Fig. 6D).

Metabolism of THC by Marihuana-Tolerant Rats

In vitro Metabolism

Although the body-weight curves indicated that the marihuana-treated rats were tolerant to M.E.-I, the metabolism of THC *in vitro* was not altered. The amount of THC metabolized (initial THC concentration, 128 μM) during incubation periods of 5, 10, 15, and 30 min was the same for olive-oil control and marihuana-tolerant rats, as determined 40 h after the end of a 28-day treatment period (Fig. 7). The TLC pattern of THC metabolites was also not altered by the chronic treatment. Furthermore, the metabolism of THC *in vitro* was the same in the treated and control groups when the initial THC concentration was reduced to 51 μM, indicating that neither the apparent K_m nor V_{max} for THC metabolism was altered by the chronic marihuana treatment.

Similarly there was no difference between control and treated rats when THC metabolism *in vitro* was determined at 21.5, 63, or 84 h following the chronic treatment. In addition, the

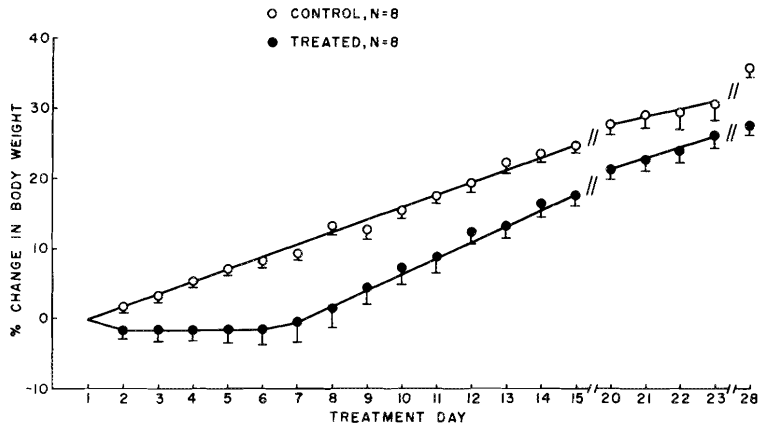

FIG. 5. Effect of chronic treatment of rats with M.E.-I on body weight. The initial M.E.-I dose, providing THC, 5 mg/kg, was increased on day 11 to provide THC, 7.5 mg/kg, and on day 21 to 10 mg/kg; points and bars represent means and S.E.M.

TABLE 2. Analysis of variance for the influence of the order of olive oil and M.E.-II treatment on ethanol-induced sleeping time

		Olive oil + ethanol		M.E.-II + ethanol	
		*F**	*F*	*F*	*F*
Source of variation	*DF*	(Days 1 and 2)	(Days 13 and 14)	(Days 1 and 2)	(Days 13 and 14)
Between orders of treatment	1, 24	2.99	0.31	0.95	1.41
Orders × groups	2, 24	1.28	1.07	1.11	0.27
Orders × groups × doses	6, 24	0.82	1.05	2.16	1.49

*All *F* values indicate absence of statistically significant differences between data groups compared; $P > 0.05$ in all cases.

TABLE 3. Analysis of variance for the effect of olive oil and M.E.-II on ethanol-induced sleeping time

Source of variation	*DF*	*F* (before chronic treatment)	*F* (after chronic treatment)
Olive oil *vs.* M.E.-II:			
Ad libitum control	1, 24	34.81*	48.20*
Pair-fed control	1, 24	33.52*	26.94*
Treated	1, 24	20.40*	11.33**

*$P < 0.001$.
**$P < 0.005$.

weights of liver, lungs, kidneys, and brain relative to body weight, and the 10 KS protein content of the livers were not changed in the marihuana-tolerant rats when measured at any time interval following the end of chronic treatment.

Biliary Excretion of THC

Although the M.E.-II treated rats were shown to be tolerant to M.E.-II and thus to THC (Fig. 6) the biliary excretion of THC, studied at 24–26 h following the end of chronic treatment, was not changed (Fig. 8). The rates of ^{14}C excretion in the bile of treated rats were not significantly different from the rates in the pair-fed or *ad libitum*-fed control rats at any time during the 3-h experimental period. Furthermore, the THC-metabolite pattern in the 10–20 min bile samples (*i.e.* at the time of highest biliary-excretion rate) was the same

TABLE 4. Analysis of variance for the influence of chronic-marihuana treatment on the prolongation of ethanol-induced sleeping time by M.E.-II

Source of variation	*DF*	*F* (M.E.-II + ethanol sleep: end of treatment)	*F* (M.E.-II + ethanol sleep: end minus start of treatment)
Between treatments	2, 36	9.03*	13.51*
Treated *vs.* pair-fed	1, 24	10.84**	13.04*
Treated *vs. ad libitum*	1, 24	9.09***	35.62*
Pair-fed *vs. ad libitum*	1, 24	0.47 n.s.	2.05 n.s.

*$P < 0.001$.
**$P < 0.005$.
***$P < 0.01$.
NOTE: n.s., no significant difference.

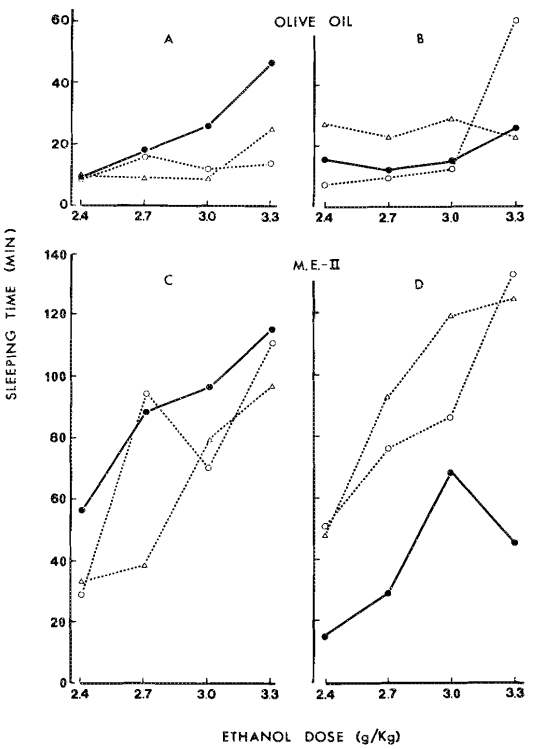

FIG. 6. Effect of olive oil and M.E.-II on ethanol-induced sleeping time in rats before and after chronic treatment. Treated chronically with M.E.-II, ●; pair-fed control, ○; *ad libitum*-fed control, △. Values represent means; N = four per point. A and B, olive oil + ethanol tests; C and D, M.E.-II + ethanol. A and C, tests before chronic treatment; B and D, after chronic treatment.

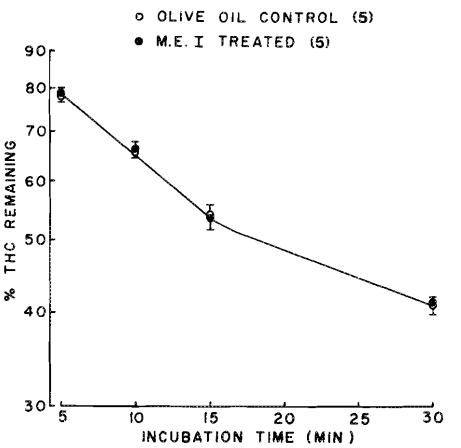

FIG. 7. THC metabolism *in vitro* 40 h following the last dose of a 28-day chronic treatment of rats with M.E.-I (see text for details of treatment and assay conditions). Initial THC concentration, 100 μg/2.5 ml. Points and bars represent means and S.E.M.; N = five per group.

in all three groups of rats (Table 5). The relative proportions of the various metabolites might well change over time, as the residual concentrations of THC and early metabolites in the body fall.

There was no difference in the total amounts of ^{14}C excreted in 3 h by the treated (51.55 ± 0.97%) and pair-fed control (51.66 ± 2.54%) groups. However, the *ad libitum*-fed controls excreted a significantly greater amount (55.95 ± 1.92%, $P < 0.05$) than the treated animals. The difference between the two control groups was not significant because of the relatively large variation in the total amounts excreted. The rates of bile flow, ranging from 21 to 24 mg/min, did not differ during any time interval in the three groups.

In agreement with the absence of any difference in the rates of ^{14}C excretion in the bile, there was also no difference among the rates of ^{14}C disappearance from the blood of rats in

TABLE 5. Pattern of THC metabolites in the bile of rats tolerant to marihuana*

Animal treatment	Radioactivity in each fraction as percentage of total in sample-TLC zones†					
	Non-extractable	I	II	III	IV	V
Ad libitum-fed control, $N = 4$	20.4 ± 1.5	54.0 ± 3.7	14.4 ± 1.3	8.6 ± 2.7	2.8 ± 1.0	0
Pair-fed control, $N = 5$	19.6 ± 0.8	50.9 ± 2.7	15.9 ± 1.9	9.5 ± 0.8	4.0 ± 0.3	0
M.E.-II treated, $N = 5$	16.5 ± 1.7	56.4 ± 2.2	13.8 ± 1.0	9.8 ± 1.4	3.4 ± 0.7	0

*Bile samples collected in the 10–20 min interval following THC injection.
†R_f of zones: I = 0–0.13; II = 0.14–0.22; III = 0.23–0.60; IV (7-OH-THC) = 0.61–0.70; V (THC) = 0.85–0.95.
NOTE: Values represent means ± S.E.M.

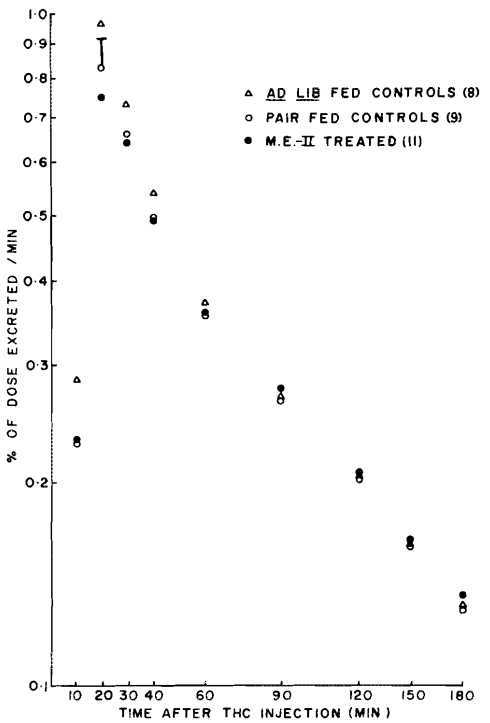

FIG. 8. Effect of chronic treatment of rats with M.E.-II on the rate of biliary excretion of THC. Points represent mean values, with *N* for each group shown in parentheses. Vertical bar at the 20-min point for pair-fed controls indicates the largest S.E.M. found for any of the plotted points.

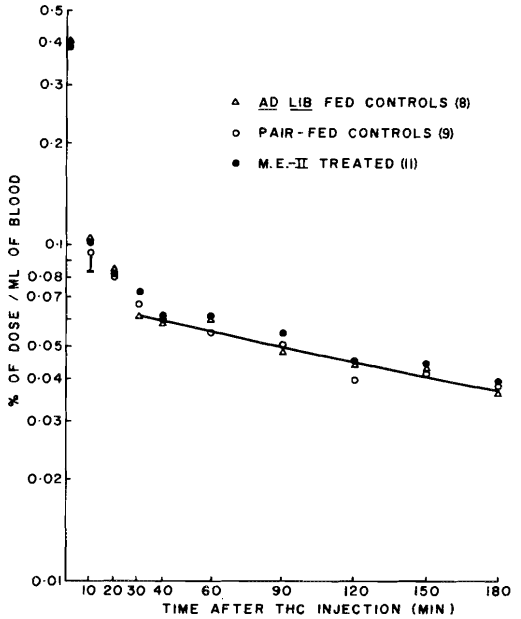

FIG. 9. Effect of chronic treatment of rats with M.E.-II on the disappearance of ^{14}C from the blood. Points represent mean values; *N* for each group shown in parentheses; bar indicates largest S.E.M. of any plotted point.

the three groups, as determined by linear-regression analysis from 30 to 180 min (Fig. 9). A single composite regression line was therefore drawn for the pooled results. The mean $t_{\frac{1}{2}}$ of this disappearance phase was 3.5 h, which agrees with previous findings in animals (Agurell *et al.* 1970; Klausner and Dingell 1971; Fehr and Kalant 1974). The apparent volumes of distribution were not altered by the treatment with M.E.-II.

The tissue distribution of total ^{14}C at 3 h was also not significantly different in the 3 groups (Table 1). The total ^{14}C present in the urine and the four tissues examined amounted to only about 4–6% of the dose, whereas about 52% was excreted in the bile, as noted above. The remaining 42–44% was presumably distributed in the remaining viscera, muscle, and body fat, as reported elsewhere (Willinsky *et al.* 1974). In addition, the relative concentrations of the THC and its metabolites in the examined tissues were the same in the two control and treated groups of animals. THC accounted for 60–70% of the radioactivity in the livers and brains, and 50–60% in the lungs. Only highly polar metabolites were detected in the urine. The kidneys were not analyzed for their metabolite content.

The ethanol treatments on the two test days immediately before the chronic-treatment period produced a small decrease in the body weight of all three groups of animals. Therefore a selective decrease in body weight due to marihuana treatment (Fig. 5) was partially masked in these experiments, although the *ad-libitum* control rats did gain weight more rapidly than the other two groups during the first 4–5 days of chronic treatment.

The biliary excretion, disappearance from the blood, and tissue distribution of ^{14}C-THC and its metabolites were found to be the same at 48 and 72 h following the last dose in the chronic treatment as at 24 h after.

Discussion

Pretreatment with phenobarbital resulted in an increase in the total capacity of rats to

metabolize THC as determined *in vitro* and *in vivo*. Experiments carried out *in vitro* demonstrated that the rate of formation of THC metabolites more polar than 7-OH-THC was greatly increased by the treatment. Other investigators (Nilsson *et al.* 1970; Wall 1971; Gill *et al.* 1973) have attempted to increase the yield of 7-OH-THC by incubating THC for up to 3 h with phenobarbital-induced rat-liver preparations. However, the present results indicate that the amount of 7-OH-THC found in liver-incubation mixtures from phenobarbital-treated rats is significantly lower than in those from controls. Furthermore, in studies with control rats in which incubations were continued up to 160 min, 7-OH-THC was detected at greatly reduced levels at all times after 20 min of incubation (Siemens and Kalant 1974). The probable explanation is that 7-OH-THC is rapidly converted to more polar metabolites.

Phenobarbital pretreatment produced an initial 2.7-fold increase in the rate of total ^{14}C biliary excretion. This change in excretion rate probably reflects a true increase in the rate of production of excretable polar THC metabolites, since it could not be accounted for by a slight increase in the rate of bile flow. That the metabolites detected in the bile were apparently the same in both groups of rats may indicate that the THC metabolites are excreted as soon as a certain degree of polarity is achieved. Therefore, although the total amount of drug excreted per unit time may be increased by phenobarbital induction, the metabolite pattern need not be altered.

The increased rate of disappearance of total ^{14}C from the blood of the phenobarbital group, as well as the decreased ^{14}C content in the livers and kidneys at 3 h, also reflect the greater rate of elimination of the drug. These results demonstrated that the *in-vitro* and *in-vivo* assay techniques for THC metabolism were indeed adequate to detect an induction of metabolism.

Chronic treatment of rats with the marihuana extracts resulted in the development of tolerance to the marihuana, as shown by two different indices. Rats used for the *in-vitro* studies became tolerant to an effect of M.E.-I, which produced an initial loss of body weight. This type of tolerance to THC and cannabis extracts has been reported by others (Pirch *et al.* 1970; Rating *et al.* 1972).

Enhancement of the depressant effect of ethanol in rats by acute treatment with M.E.-II also agrees with previous reports by others (Forney and Kiplinger 1971; Kalant and LeBlanc 1974). The rats that were treated with M.E.-II for 13–14 days became tolerant to the M.E.-II induced prolongation of ethanol sleeping time, whereas the control groups did not.

The lack of change in rate of THC metabolism, both *in vitro* and *in vivo*, as well as in residual-radioactivity levels in the tissues, in the tolerant animals as compared with controls, stands in marked contrast to the change in THC metabolism following phenobarbital induction. The *in vitro* studies were carried out at times ranging from 21.5 to 84 h after the last dose of M.E.-I to test for any possible inhibitory effect of residual drug from the chronic treatment on the metabolizing enzymes. An inhibitory effect of M.E.-I on pentobarbital metabolism had been found previously (Siemens *et al.* 1974), but did not appear to be exerted on THC metabolism in the present study. This does not completely exclude the possibility of induction of drug-metabolizing enzymes by cannabis, since any induced increase might have disappeared at least as rapidly as the inhibitory component of M.E.-I. However, the M.E.-II did not inhibit pentobarbital metabolism in earlier work (Siemens *et al.* 1974), and no induction of THC metabolism was found in animals treated with it in the present study. This is consistent with the absence of any increase in liver size or hepatic-microsomal protein content. The slightly higher rates of ^{14}C excretion in the bile of *ad libitum*-fed controls may indicate that the diet influenced the rate of THC metabolism slightly.

Therefore the tolerance observed apparently did not occur as a result of a more rapid removal of either THC or 7-OH-THC from their site of action. Recently Kupfer *et al.* (1973) have reported a similar conclusion: chronic treatment of rats with THC or a marihuana extract had no effect on hepatic-microsomal metabolism of THC or other drugs *in vitro*.

In contrast, Ho *et al.* (1973) have suggested that oral treatment of rats for 4 weeks with THC (1 mg/kg) in a 4% Tween-80 and saline suspension increased the rate of hepatic-microsomal metabolism of THC, whereas THC metabolism in the lung was not altered. However, the interpretation of their results is question-

able. First, apparently the control animals received only saline instead of the THC vehicle. Therefore Tween-80 may have induced the metabolism in the treated group. Moreover, the hepatic-metabolic rates obtained for the chronically treated animals were not compared with the rates obtained for the socalled control animals, but instead with rates determined for animals that had been treated acutely with THC. It is known that a single dose of THC can decrease food and water intake in rats (Rating *et al.* 1972), and that fasting can decrease the rate of hepatic drug metabolism (Gillette 1971). Therefore the apparent difference in the rates of THC metabolism between the two groups may be due to an acute inhibition of metabolism in control rats rather than an inductive effect in the chronically treated animals.

Furthermore, Ho *et al.* (1973) apparently used an incubation period of 1–3 h in the presence of an NADPH-generating system containing 1×10^{-4} *M* NADP. Under these conditions, they observed an overall metabolism of only 10–17% of the added THC, which is considerably below that found under optimal conditions (Siemens and Kalant 1974). Even in the latter case, THC metabolism remained linear with time for only 30 min or less. If the rates of metabolism of two samples are compared during the non-linear phase, it is difficult to draw a valid conclusion.

Neither THC metabolism (McMillan *et al.* 1973) nor THC distribution (Dewey *et al.* 1973) was altered in pigeons tolerant to THC compared with non-tolerant controls. Our findings are in agreement with these, and at variance with the suggestion that chronic use of marihuana may increase the rate of THC metabolism in humans (Lemberger *et al.* 1971). Therefore tolerance to marihuana in rats may be considered "functional" rather than "dispositional," as defined by Kalant *et al.* (1971).

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AGURELL, S., NILSSON, I. M., OHLSSON, A., and SANDBERG, F. 1970. On the metabolism of tritium-labelled Δ^1-tetrahydrocannabinol in the rabbit. Biochem. Pharmacol. **19**, 1333–1339.

C.I.N.M.U.D. 1972. Cannabis and its effects. *In* Cannabis, a report of the commission of inquiry into the non-medical use of drugs. G. LeDain, Chairman. Information Canada, Ottawa, Canada. pp. 119–125.

CONNEY, A. H. 1967. Pharmacological implications of microsomal enzyme induction. Pharmacol. Rev. **19**, 317–366.

DEWEY, W. L., MCMILLAN, D. E., HARRIS, L. S., and TURK, R. F. 1973. Distribution of radioactivity in brain of tolerant and non-tolerant pigeons treated with ^{3}H-Δ^9-tetrahydrocannabinol. Biochem. Pharmacol. **22**, 399–405.

FORNEY, R. B., and KIPLINGER, G. F. 1971. Toxicology and pharmacology of marihuana. Ann. N.Y. Acad. Sci. **191**, 74–82.

GILL, E. W., JONES, W., and LAWRENCE, D. K. 1973. Contribution of the metabolite 7-hydroxy-Δ^1-tetrahydrocannabinol towards the pharmacological activity of Δ^1-tetrahydrocannabinol in mice. Biochem. Pharmacol. **22**, 175–184.

GILLETTE, J. R. 1971. Factors affecting drug metabolism. Ann. N.Y. Acad. Sci. **179**, 43–66.

HO, B. T., ESTEVEZ, V. S., and ENGLERT, L. F. 1973. Effect of repeated administration on the metabolism of Δ^9-THC in rats. Res. Commun. Chem. Pathol. Pharmacol. **5**, 215–218.

HO, B. T., ESTEVEZ, V., ENGLERT, L. F., and MCISAAC, W. M. 1972. Δ^9-Tetrahydrocannabinol and its metabolites in monkey brains. J. Pharm. Pharmacol. **24**, 414–416.

JÄRBE, T. U. C., and HENRIKSSON, B. G. 1973. Acute effects of two tetrahydrocannabinols (Δ^9-THC and Δ^8-THC) on water intake in water deprived rats: implications for behavioral studies on marihuana compounds. Psychopharmacologia, **30**, 315–322.

KALANT, H., and LEBLANC, A. E. 1974. Effect of acute and chronic pretreatment with Δ^1-tetrahydro-cannabinol on motor impairment by ethanol in the rat. Can. J. Physiol. Pharmacol. **52**, 291–297.

KALANT, H., LEBLANC, A. E., and GIBBINS, R. J. 1971. Tolerance to, and dependence on, some non-opiate psychotropic drugs. Pharmacol. Rev. **23**, 135–191.

KATER, R. M. H., ROGGIN, G., TOBON, F., ZIEVE, P., and IBER, F. L. 1969. Increased rate of clearance of drugs from the circulation of alcoholics. Am. J. Med. Sci. **258**, 35–39.

KHANNA, J. M., and KALANT, H. 1970. Effect of inhibitors and inducers of drug metabolism on ethanol metabolism *in vivo*. Biochem. Pharmacol. **19**, 2033–2041.

KLAUSNER, H. A., and DINGELL, J. V. 1971. The metabolism and excretion of Δ^9-tetrahydrocannabinol in the rat. Life Sci. **10**, Pt. I, 40–59.

KUPFER, D., LEVIN, E., and BURSTEIN, S. H. 1973. Studies on the effects of Δ^1-THC and DDT on the hepatic microsomal metabolism of Δ^1-THC and other compounds in the rat. Chem. Biol. Interact. **6**, 59–66.

LEMBERGER, L., SILBERSTEIN, S. D., AXELROD, J., and KOPIN, I. J. 1970. Marihuana: studies on the disposition and metabolism of delta-9-tetrahydrocannabinol in man. Science, **170**, 1320–1322.

LEMBERGER, L., TAMARKIN, N. R., AXELROD, J., and KOPIN, I. J. 1971. Delta-9-tetrahydrocannabinol: metabolism and disposition in long-term marihuana smokers. Science, **173**, 72–74.

LINEWEAVER, H., and BURK, D. 1934. The determination of enzyme dissociation constants. J. Am. Chem. Soc. **56**, 658–666.

MANNING, F. J., MCDONOUGH, J. H., JR., ELSMORE, T. F., SALLER, C., and SODETZ, F. J. 1971. Inhibition of normal growth by chronic administration of Δ^9-tetrahydrocannabinol. Science, **174**, 424–426.

MCMILLAN, D. W., DEWEY, W. L., and HARRIS, L. S. 1971. Characteristics of tetrahydrocannabinol tolerance. Ann. N.Y. Acad. Sci. **191**, 83–99.

MCMILLAN, D. W., DEWEY, W. L., TURK, R. F., HARRIS, L. S., and MCNEIL, J. H., JR. 1973. Blood levels of ^{3}H-Δ^9-tetrahydrocannabinol and its metabolites in tolerant and nontolerant pigeons. Biochem. Pharmacol. **22**, 383–397.

NILSSON, I. M., AGURELL, S., NILSSON, J. L. G., OHLSSON, A., SANDBERG, F., and WAHLQVIST, M. 1970. Δ^1-Tetrahydrocannabinol: structure of a major metabolite. Science **168**, 1228–1229.

FEHR, K. O., and KALANT, H. 1974. Fate of ^{14}C-Δ^1-THC in rat plasma after intravenous injection and smoking. Eur. J. Pharmacol. **25**, 1–8.

PATON, W. D. M., and PERTWEE, R. G. 1973. The pharmacology of cannabis in animals. *In* Marihuana: chemistry, pharmacology, metabolism and clinical effects. *Edited by* R. Mechoulam. Academic Press Inc., New York and London. pp. 191–285.

PIRCH, J. H., COHN, R. A., OSTERHOLM, K. C., and BARRATT, E. 1972. Tolerance to the effects of marijuana on behaviour and body weight of rats. Fifth International Congress on Pharmacology. p. 183, Abstr. 1096.

RATING, D., BROERMAN, I., HONECKER, H., KLUWE, S., and COPER, H. 1972. Effect of subchronic treatment with (−)-Δ^8-*trans*-tetrahydrocannabinol on food intake, body temperature, hexobarbital sleeping time and hexobarbital elimination in rats. Psychopharmacologia, **27**, 349–357.

RUBIN, E., and LIEBER, C. S. 1968. Hepatic microsomal enzymes in man and rat: induction and inhibition by ethanol. Science, **162**, 690–691.

SIEMENS, A. J., and KALANT, H. 1974. Metabolism of Δ^1-tetrahydrocannabinol by the rat *in vivo* and *in vitro*. Biochem. Pharmacol. In press.

SIEMENS, A. J., KALANT, H., KHANNA, J. M., MARSHMAN, J., and HO, G. 1974. Effect of cannabis on pentobarbital-induced sleeping time and pentobarbital metabolism in the rat. Biochem. Pharmacol. **23**, 477–488.

WALL, M. E. 1971. The *in vitro* and *in vivo* metabolism of tetrahydrocannabinol (THC). Ann. N.Y. Acad. Sci. **191**, 23–39.

WILLINSKY, M. D., KALANT, H., MERESZ, O., ENDRENYI, L., and WOO, N. 1974. Distribution and metabolism *in vivo* of ^{14}C-Δ^1-tetrahydrocannabinol in the rat. Eur. J. Pharmacol. **25**, 106–119.