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THE PHARMACOKINETIC FATE OF CANNABIDIOL AND ITS RELATIONSHIP TO BARBITURATE SLEEP TIME

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Abstract—Cannabidiol (CBD) is a known inhibitor of a number of hepatic drug metabolism reactions. Its pharmacokinetics in the liver were studied to determine the relationship between the amount of CBD in this organ and the effect on barbiturate sleep time, which has been shown by others to reflect an inhibition of drug metabolism. A methanol extract of liver was subjected to thin-layer chromatography which yielded three distinct fractions: CBD, the monohydroxylated metabolites and a relatively polar, unidentified fraction. The quantitative analysis of these fractions, as a function of time after drug administration, indicated that CBD is metabolized rapidly: the apparent half life is only about 52 min, which is approximately the same value obtained for the monohydroxylated metabolites; both of these fractions have virtually disappeared from the liver at 4 hr, at which time a significant effect on sleep time still persists. In contrast, the unidentified metabolite fraction contains a substantial amount of cannabinoid throughout the course of the effect on sleep time. These data suggest that the inhibition of hepatic drug metabolism is not caused by either CBD or one of its monohydroxylated metabolites; rather, the effect correlates with the persistence of more polar metabolites.

It was Loewe [1] who originally observed that cannabis extracts prolonged barbiturate sleep time and he suggested that this effect was due to the cannabidiol (CBD) content of cannabis. Later, Paton and Pertwee [2] confirmed the observations of Loewe; on the basis of the *in vitro* effects of CBD on drug metabolism by liver microsomes, they proposed that the drug prolongs sleep time by blocking barbiturate metabolism. Similarly, Fernandes *et al.* [3] and Siemens *et al.* [4] also reported that CBD *in vitro* blocks hepatic drug metabolism; in addition, the latter group of investigators published direct evidence that CBD blocks pentobarbital metabolism *in vivo* and that the inhibition of metabolism can account for the prolongation of sleep time, which in the rat persists for as long as 63 hr after a single exposure to the drug [5].

The mechanism by which CBD blocks drug metabolism is undefined; however, the drug does form a type 1 binding spectrum with cytochrome P-450 [3, 6]. The spectral dissociation constant is relatively low for CBD, but whether this accounts for the very long duration of action is not known [5]. The intent of the following experiments was to describe the inhibitory mechanism by means of a pharmacokinetic analysis of CBD and its metabolites in the liver after both single and repeated administration.

MATERIALS AND METHODS

CBD administration and hexobarbital sleep time determination. Male mice (Charles River ICR), about 4- to 5-weeks-old, were used in all the experiments. As described previously [7], CBD was prepared for intraperitoneal injection by adding Tween 80 to an alcoholic stock solution of the cannabinoid followed by thorough mixing and evaporation of the alcohol in a stream of N_2. The CBD–Tween 80 residue was dispersed by ultrasound (Branson Sonifier S-75) in an isotonic saline solution; the final drug mixture contained approximately 3%, by volume, of Tween 80. Most of the experiments involved either single or repeated anticonvulsant doses of CBD, 120 mg/kg [8]; in the metabolite distribution experiments, [^{3}H]CBD (80 μCi/mg) was added to each injection and the animals received 850 μCi [^{3}H]CBD/kg body weight. For the pharmacokinetic analyses, the amounts of CBD and its principal metabolites in the liver were determined at various times after acute administration of the drug. In addition, the influence of repeated daily CBD administration on the hepatic cannabinoid content was measured in animals treated with single doses for 1, 2, 4 and 8 days; the cannabinoids were determined 24 hr after the final dose was administered. All animals were killed by cervical dislocation.

Hexobarbital sleep times were determined at various intervals after the administration of CBD or vehicle; sleep time was measured from the loss to the recovery of the righting reflex, following a 100 mg/kg dose of the barbiturate. Hexobarbital was prepared for intraperitoneal injection in normal saline; the injection volume was 0.1 ml/20 g body wt. Brain hexobarbital concentrations were determined spectrophotometrically by the method of Vesell [9].

The relatively low specific activity of the injected [^{3}H]CBD (7 μCi/mg), combined with the rapid metabolism of CBD, presented a problem in the quantitation of CBD and some of its metabolites; therefore, in order to increase the amount of radioactivity chromatographed, the tissue extracts were concentrated as described below.

Extraction procedure for tissue cannabinoids. A portion of the liver (about 0.5 g) from each mouse was homogenized in 4 vol. methanol with an all glass, conical homogenizer (Kontes Duall) and centrifuged at room temperature for 10 min at 1000 *g*. The supernatant fluid was transferred to a glass centrifuge tube and the pellet washed twice with 2 vol. methanol by resuspension and centrifugation. The supernatant fractions obtained from the washes were added to the first methanol supernatant fluid, and the combined supernatant

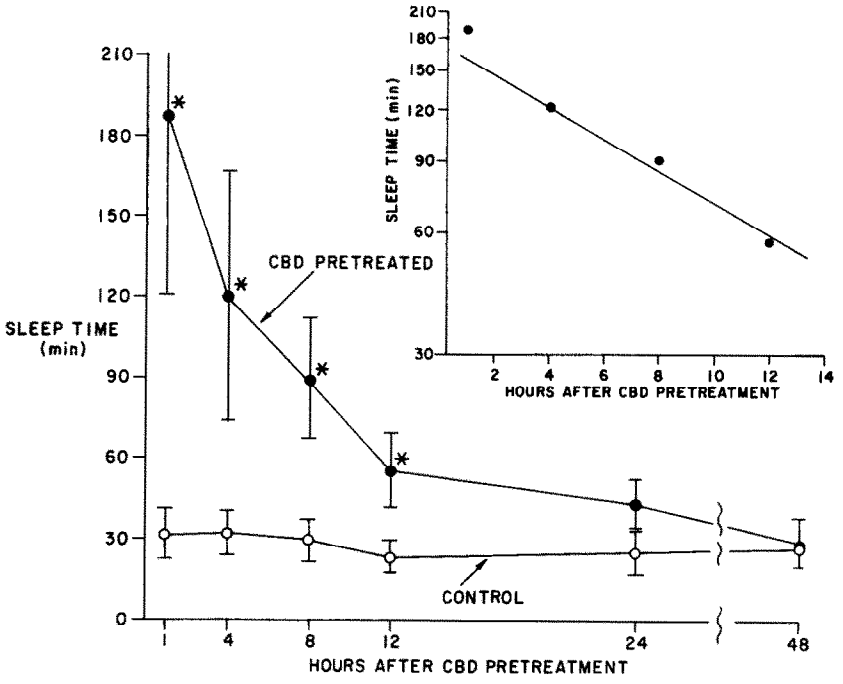

Fig. 1. Time course of the effect of CBD on hexobarbital sleep time. Sleep time was measured after the administration i.p. of 100 mg/kg of hexobarbital 1, 4, 8, 12, 24 and 48 hr after the i.p. administration of 120 mg/kg of CBD. Each value shown is the mean ± one S.D. of a group of twenty animals. The starred values are significantly different ($P<0.05$) from their controls, as determined by analysis of variance [10, 11]. The inset represents a semilogarithmic plot of the significantly different sleep-time values. The half-life of the effect (7 hr) was calculated from the slope of the line (-0.045) determined by linear regression analysis of the data; the correlation coefficient for the line is 0.83. The slope of the line was significantly different from zero as determined by a *t*-test ($P<0.001$).

fluids were subsequently chilled in an ice-bath which resulted in the formation of a precipitate; the sample was then centrifuged for 10 min at 1000 *g* at 0–5°. The resultant supernatant fluid was transferred to glass culture tubes (12 × 75 mm), and the pellet was redissolved in methanol (at 24°) and transferred to a scintillation vial. The supernatant fluid was evaporated to approximately 25 per cent of its original volume with N_2, chilled in an ice-bath, and centrifuged for 10 min at 1000 *g* at 0–5° to remove a second precipitate which formed in the cold methanol. The supernatant fraction was transferred to a clean culture tube and evaporated to dryness with N_2; the pellet was transferred to a scintillation vial as described for the first precipitate. The supernatant residue was extracted twice with 100 μl chloroform which was transferred to glass microtubes (5 mm i.d. × 75 mm). The 200 μl chloroform was evaporated with N_2 and 200 μl methanol added. An aliquot of this final concentrated extract was taken for the determination of the amount of radioactivity applied to the thin-layer chromatography (t.l.c.) sheets, another aliquot was applied to a t.l.c. sheet, and the remaining solution was washed into a scintillation vial to measure the amount of radioactivity not spotted on the t.l.c. From this information, the recovery of radioactivity in the initial homogenate and methanol extract could be estimated. An aliquot (100 μl) of the initial homogenate and the entire pellet derived from the centrifugation of the homogenate were both solubilized in 2 ml of 2 M piperidine. All of the samples, homogenate and pellet digest, the two pellets precipitated from cold methanol, and the various aliquots of the final methanol concentrate, were counted in 15 ml Aquasol (New England Nuclear, Boston, MA).

Thin-layer chromatography of tissue extracts. The concentrated tissue extracts were applied to 20 × 20 cm Eastman silica gel sheets, without fluorescent indicator, and developed in hexane and acetone (4:3). The developed chromatograms were sprayed with a 0.1% solution of Fast Blue B in 70% ethanol for visualization of the cannabinoids, and then cut into 1 × 2 cm strips, which were placed in a scintillation vial containing Aquasol (New England Nuclear). Samples were counted in an Amersham/Searle PDS/3 Isocap scintillation spectrometer and the radioactivity measured was corrected for quench and machine efficiency.

RESULTS

The results shown in Fig. 1 illustrate the effect of CBD on hexobarbital sleep time as determined at various intervals following acute administration of the drug (120 mg/kg). Sleep time was increased significantly for as long as 12 hr after CBD pretreatment. During this period, the drug effect appeared to decrease exponentially and as would be expected from an exponential decrease, the variability in the CBD effect was greater at the earlier intervals. To avoid the variability observed at 1 hr and to keep the experiments within a practical time limit, sleep time in subsequent acute

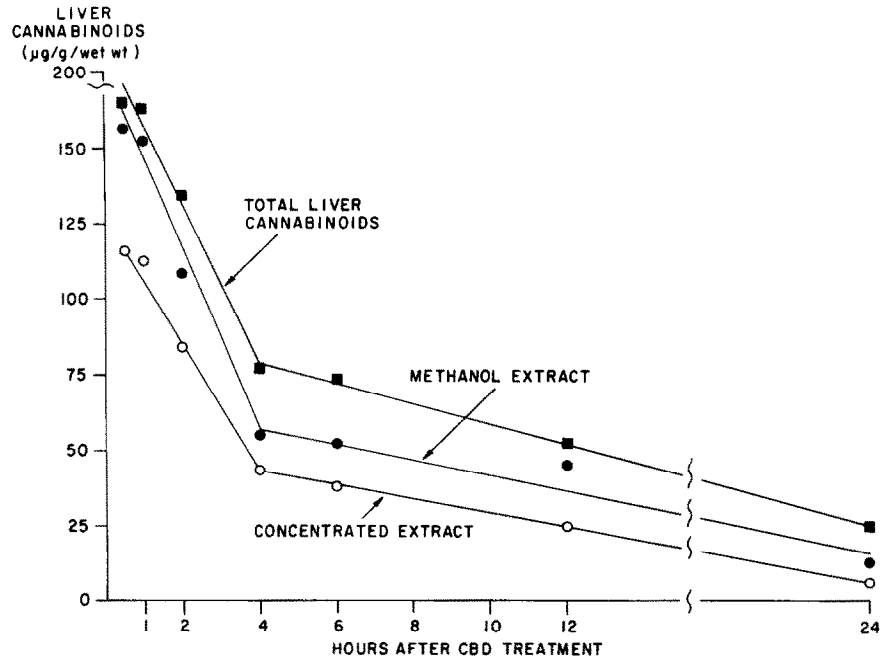

Fig. 2. Time course of the liver cannabinoids following acute [^{3}H]CBD administration. Total liver cannabinoids were determined in the liver homogenate; the methanol extract represents the cannabinoids recovered in methanol from the liver homogenate; the concentrated extract is the recovery in the methanol extract after reducing the volume of the methanol to about $\frac{1}{10}$ of the original extract. The values shown at each time are the means of six separate experiments.

studies was determined 4 hr after pretreatment with CBD. The insert shown in Fig. 1 is a semilogarithmic plot of the significantly different sleep times after CBD pretreatment. The slope of the line was calculated to determine the half-life of the effect, which was about 7 hr; compare, below, this value with the apparent half-lives of CBD and its metabolites in the liver.

The prolongation of sleep time depicted in Fig. 1 has been attributed usually to an inhibitory effect of CBD on the hepatic metabolism of the barbiturate, rather than to a central effect of CBD. The evidence for such an interpretation was derived, however, from studies involving much lower doses of CBD than were used in the present work (10 mg/kg vs 120 mg/kg) [2]. In the present investigation, the concentrations of hexobarbital in the brains of eight control mice and eight mice pretreated with 120 mg/kg of CBD were determined in awakening animals. The mean and standard deviation values for the control and CBD-treated mice were 36.8 ± 5.6 and 36.5 ± 7.6 μg/g of brain respectively. The essentially identical brain concentrations in the control and CBD-pretreated animals suggest that the CBD-caused prolongation of sleep time, even using these relatively high doses, is still the consequence of a reduced rate of metabolism of hexobarbital and is not a centrally mediated effect.

In the next series of experiments, [^{3}H]CBD was used to determine the concentration of cannabinoids in the liver as a function of time after acute CBD administration. Initially, total tissue cannabinoids were compared with the totals recovered in a methanol extract. The cannabinoid concentration in these two preparations is illustrated in Fig. 2 as a function of time after acute drug administration. At the early times, the methanol extract contained a very high percentage of the total tissue cannabinoids, but the recovery decreased with time, and at 24 hr, for example, the methanol extract contained only about 50 per cent of the total tissue cannabinoids. This decline in recovery as a function of time was construed to reflect the metabolic conversion of CBD to

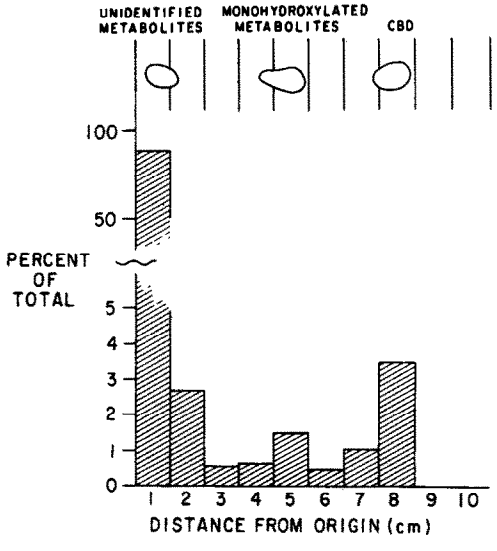

Fig. 3. Chromatographic analysis of a concentrated methanol liver extract 1 hr after [^{3}H]CBD administration. At the top of the figure is a reproduction of the actual chromatogram; the histogram represents the quantitative distribution of the cannabinoids in each 1-cm section of the chromatogram.

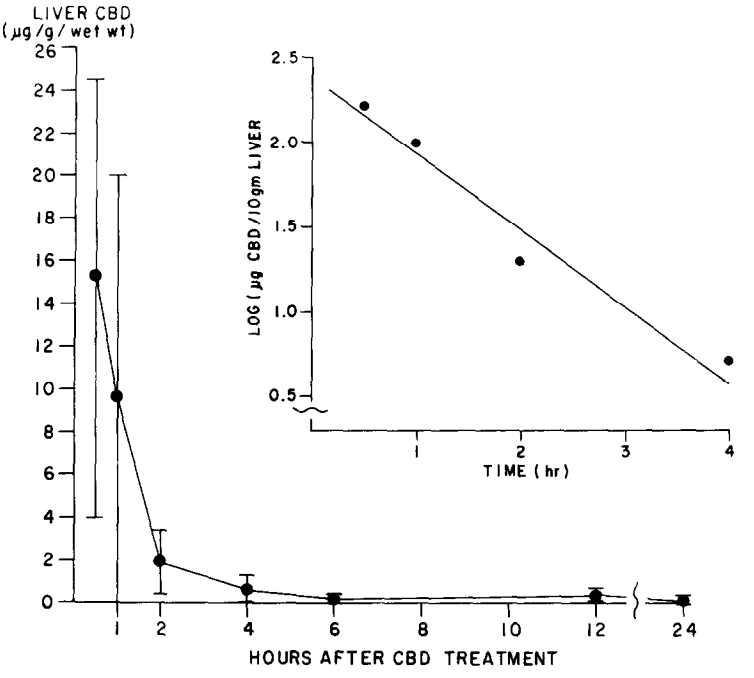

Fig. 4. Time course of CBD in the liver following acute administration. [^{3}H]CBD, 120 mg/kg, was administered i.p. The value for each time interval is the mean ± one S.D. of a group of six animals. The insert represents a semilogarithmic plot of the values for the first 4 hr. The CBD half-life (52 min) was calculated from the slope of the line (-0.35) determined by linear regression analysis of the data; the correlation coefficient for the line is 0.76. The slope of the line is significantly different from zero as determined by a t-test ($P<0.001$).

non-methanol-extractable polar metabolites. In addition, the data indicate that there is a fairly rapid decrease in total liver cannabinoids.

By reducing the volume of the methanol extract in order to obtain the concentrated extract (also shown in Fig. 2), approximately 5 and 15 per cent of the recovered cannabinoids were lost in the cold methanol precipitates. Despite these losses, the concentration of radioactivity was 5–10 times greater in the concentrated than in the initial methanol extract, a condition which facilitated the chromatography and subsequent quantitation of the cannabinoids.

The cannabinoids in the concentrated extract were subjected to t.l.c. in order to isolate and quantitate the CBD and metabolite content. A typical chromatogram of an extract obtained 1 hr after CBD administration is shown in Fig. 3, which includes a histogram of the percentage of the total cannabinoids isolated on each 1 cm strip from the origin. Strips 1 and 2 were pooled to represent the unidentified metabolites; strips 4, 5 and 6 comprised the monohydroxylated metabolites; and strips 7, 8 and 9 were taken to represent CBD, which was identified by co-chromatography with authentic CBD. The monohydroxylated metabolite fraction contains primarily the 11-hydroxy metabolite of CBD [12, 13]. Although no authentic 11-hydroxy-CBD was available to serve as a reference, the R_f value of this fraction corresponds to that of the primary metabolite of CBD obtained from an *in vitro* hepatic drug-metabolizing system; the R_f is also similar to that of 11-hydroxy-Δ^9-tetrahydrocannabinol when added to a concentrated extract and chromatographed. Martin *et al.* [13] reported that there are several such metabolites of CBD and that in all likelihood these are not separable by the chromatographic technique employed; hence, the fraction was termed monohydroxylated metabolites.

Figure 4 represents the concentration of hepatic CBD as a function of time. As can be seen, CBD is metabolized very rapidly; consequently, at 4 hr there is little of the drug remaining in the liver. A semilogarithmic plot of the data shown in the insert in Fig. 4 indicates that the rapid phase of the disappearance follows first-order kinetics with an apparent elimination half-life of about 52 min. Similar results were obtained in the study of the monohydroxylated metabolites (Fig. 5). Here again, by 4 hr most of these metabolites are gone from the liver. The insert in Fig. 5 is a semilogarithmic plot of the data, which also follows first-order kinetics with an apparent half-life of about 67 min.

In contrast to CBD and the monohydroxylated fractions, the unidentified metabolites do not appear to be eliminated as rapidly. In Fig. 6 it can be seen that at least some of the constituents of the unidentified fraction persist in relatively substantial amounts even at 24 hr. Their disappearance, however, does not follow simple first-order kinetics; therefore, a half-life could not be calculated from the available data. In general, the metabolites in this fraction are eliminated more slowly than are the monohydroxylated products; moreover, a quantitative comparison of these two fractions shows that the unidentified metabolites accumulate very rapidly and in relatively large amounts. For example, at 1 hr there is approximately 10 times as much material

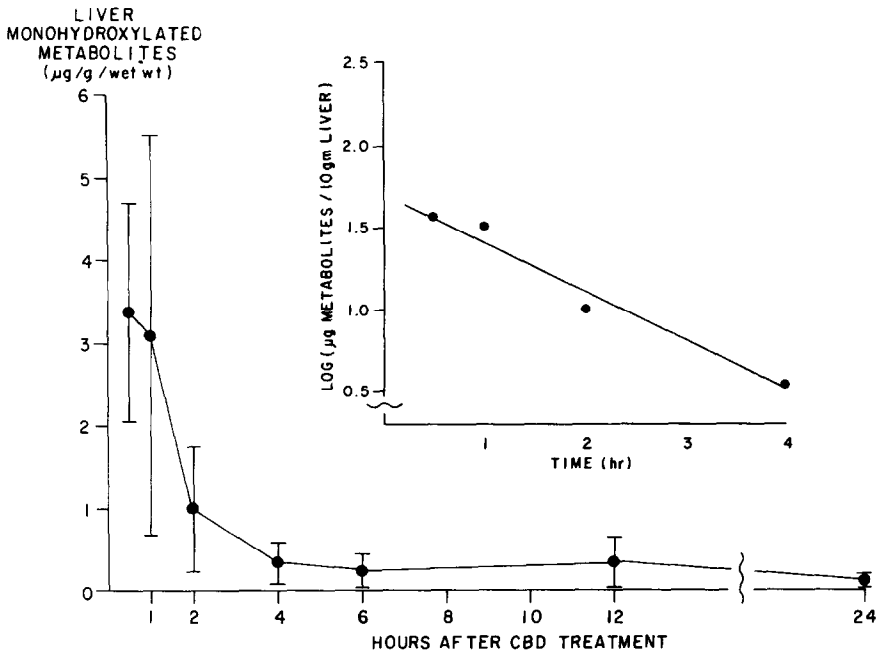

Fig. 5. Time course of the monohydroxylated metabolites of CBD in the liver following acute administration of [^{3}H]CBD. The values were obtained from the same experiments as those shown in Fig. 4. The insert represents a semilogarithmic plot of the values for the first 4 hr. The half-life of these metabolites (67 min) was calculated by using the slope of the line (-0.27) determined by a linear regression analysis of the data; the correlation coefficient for the line is 0.83. The slope of the line is significantly different from zero as determined by a t-test ($P<0.001$).

in this fraction as there is in the combined CBD and monohydroxylated fractions. Such results provide further support for the proposition that CBD is metabolized very rapidly by the liver.

Liver cannabinoid content as a function of daily [^{3}H]CBD treatment is shown in Fig. 7. These experiments were designed to measure possible accumulation of drug and metabolites with daily administration; for this purpose the cannabinoid concentrations were determined 24 hr after the final drug administration. Figure 7 includes data on total tissue cannabinoids as determined in liver homogenate, and on the three chro-

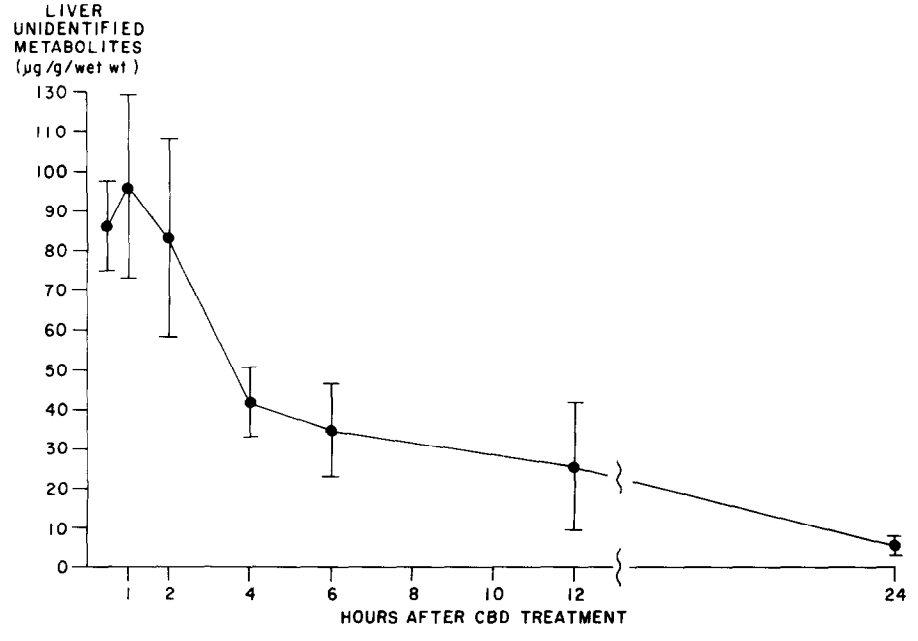

Fig. 6. Time course of the unidentified liver metabolites following acute administration of [^{3}H]CBD. The experimental values were obtained from the same experiments as those shown in Figs. 4 and 5.

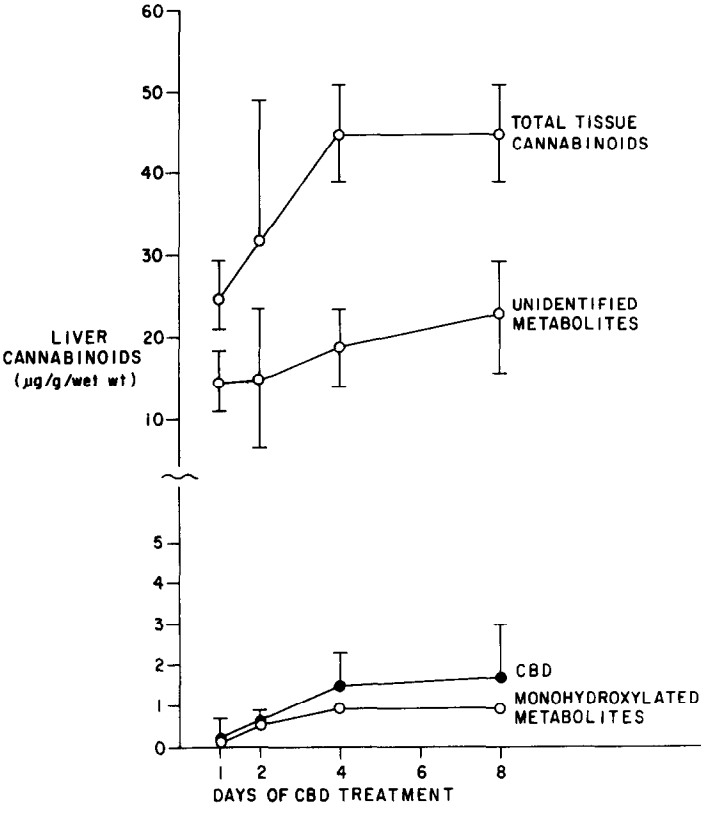

Fig. 7. Influence of repeated daily CBD administration on liver cannabinoids. [^{3}H]CBD was administered i.p. daily, 120 mg/kg. Samples were obtained 24 hr after the final drug treatment; methanol extracts were used for the chromatographic analysis. The values shown for each day are the means ± one S.D. of six separate experiments.

matographic fractions extracted from the liver with methanol, i.e. CBD, the monohydroxylated and unidentified metabolites. In these experiments, the complete extraction scheme outlined in Materials and Methods was not used; the first methanol extract was chromatographed directly without concentration. As illustrated in Fig. 7, the accumulation of total cannabinoids appears to reach a steady state after 4 days of drug administration. In contrast, there is only a small absolute increase, if any, in the three chromatographic fractions even after 8 days of drug treatment. These results are consistent with those obtained in the studies after single-dose administration (Figs. 4–6) in which relatively little drug or its methanol-extractable metabolites remained in the liver after 24 hr; therefore, little accumulation should result from daily drug administration, providing the kinetics of elimination remain unchanged. In general, the sum of these three fractions represents only about 50 per cent of the total tissue cannabinoids. A similarly low recovery was also recorded in the 24-hr data shown in Fig. 2. Once again, it is likely that the non-methanol cannabinoids are relatively polar metabolites, which accounts for the accumulation seen in the total tissue cannabinoids.

The influence of repeated daily CBD treatment on the functional status of the drug-metabolizing enzymes relative to hexobarbital sleep time is shown in Table 1. In these experiments, groups of mice were treated intraperitoneally with 120 mg/kg of CBD; the longest treatment period was 12 days. The effect of the daily treatment on sleep time was assessed 24 hr after the final CBD injection in each group. The results show that there is no effect on sleep time, even after 12 days of treatment, which suggests that there is no pharmacologically significant accumulation of drug effect with repeated daily administration.

DISCUSSION

The toxicity of CBD is a matter of concern for at least two reasons. First, CBD is a major constituent of

Table 1. Influence of daily CBD administration on hexobarbital sleep time*

Days of treatment	Sleep time	
	CBD	Vehicle
1	34 ± 10	26 ± 8
2	31 ± 10	20 ± 5
4	20 ± 6	19 ± 7
8	30 ± 8	21 ± 6
12	32 ± 7	21 ± 5

* Sleep time was assessed following the administration i.p. of 100 mg/kg of hexobarbital 24 hr after the final CBD treatment (120 mg/kg/day). The sleep-time values represent means ± one S.D., as measured in groups of twenty mice. Each group was tested for sleep time once only.

marihuana and the possible consequences of its extensive use by humans have not yet been elucidated; second, this cannabinoid has a definite therapeutic potential as an antiepileptic, requiring, therefore, chronic administration and immediately raising questions of toxicity. At present, little is known about either the acute or chronic toxicity of CBD; nevertheless, one possible toxic property has been documented: the drug was reported originally to prolong barbiturate sleep time [1] and this effect was later shown to be due to the inhibition of the hepatic, microsomal drug-metabolizing enzymes [2–4]. CBD is now recognized as a very effective inhibitor of hepatic drug metabolism; hence the possibility of drug interactions presents a definite toxic potential [2]. The purpose of the experiments described here was to initiate an investigation of the mechanism of the CBD inhibition of hepatic drug metabolism.

In the present study, the acute administration of CBD in mice produced, for a period of 12 hr, a statistically significant prolongation of sleep times, a result which is consonant with that recorded by Paton and Pertwee [2] but which is much shorter than the 63-hr duration of the effect described in rats by Siemens *et al.* [5]. Nevertheless, even a 12-hr period is longer than that reported (using identical doses in the same strain of mice) for such central effects as anticonvulsant activity and neurotoxicity [7]; these persist for only about 4 hr. In terms of anticonvulsant and neurotoxic activity, the active form may be CBD itself and not a metabolite, because the course of the drug in the brain parallels the course of both these effects (R. Karler, unpublished observations). In contrast, in the data described above, there is no quantitative correlation between the CBD content of the liver and the effect on sleep time. The results of these experiments demonstrate that CBD is metabolized very rapidly (apparent half-life in the liver of about 52 min) and is virtually eliminated from the liver by 4 hr, at which time a considerable effect on sleep time is still present. The dissociation of the course of CBD in the liver from the effect on hexobarbital sleep time suggests that the prolongation of sleep time or the inhibition of drug metabolism in the liver may not be due to CBD *per se* but rather to one or more of its metabolites. The data shown in Figs. 5 and 6 demonstrate the rapid appearance in the liver of a relatively large quantity of metabolites, which provides direct evidence that the observed decrease in tissue CBD is due, in great part, to metabolism.

The t.l.c. analyses of the methanol tissue extracts yielded three major fractions: CBD, the monohydroxylated metabolites and the more polar but unidentified metabolites. Of the metabolite fractions, the monohydroxylated products, like CBD itself, have a short apparent half-life of about 67 min and they are essentially gone from the liver in 4 hr. Again like CBD, this metabolite fraction does not correlate quantitatively with the effect on sleep time. The remaining fraction, the unidentified fraction, represents most of the methanol-extractable cannabonoids. At 4 hr, for example, when only very small amounts of the other two fractions remain, there are about 40 μg/g wet wt of cannabinoid in the unidentified fraction. Although this is less than the amount recovered at the earlier time intervals in this fraction, it is still much more than that recovered in the CBD fraction, even at the peak concentration time of 0.5 hr. Of the three t.l.c. fractions, the inhibitor of drug metabolism appears most likely to be associated with the unidentified metabolites. The possibility also exists that the inhibitor is not in the methanol extract but in the more polar metabolites remaining in the tissue after treatment with methanol. In either case, the studies described above support the hypothesis that the prolongation of barbiturate sleep time by CBD is the consequence of its metabolism, which results in the production of one or more metabolites responsible for the inhibition of drug metabolism.

The long duration of the inhibitory effect of CBD on barbiturate metabolism described by others [5] suggests that the daily administration of CBD may result in a cumulative increase in the prolongation of sleep time. In the present experiments, the daily administration of CBD for 12 days did not result in any cumulative effect in mice (Table 1). On all days tested, the sleep times of the treated animals were statistically indistinguishable from their controls. These results are generally consistent with the observation that, in the acute experiments, at 24 hr after treatment little or no effect persisted (Fig. 1). Furthermore, the determination of tissue cannabinoids after repeated daily administration of [^{3}H]CBD for as long as 8 days illustrates that there is only a small amount of cannabinoid accumulation relative to the quantities recovered initially during the acute experiments (Fig. 7). These results are also consistent with the reported rapid elimination of CBD and its metabolites (Figs. 3–6). Nevertheless, in other experimental conditions, such as those described by Siemens *et al.* [5] in which the duration of the effect in rats lasted for a few days, the daily administration of CBD could result in the attainment of a steady-state situation characterized by a cumulative increase in the effect on sleep time. In other words, whether or not daily administration will result in a cumulative effect on drug metabolism may depend upon the species; species differences in rates of drug metabolism are well established [14]. The results of daily administration of CBD for as long as 12 days also demonstrate that withdrawal from repeated CBD treatment does not alter hexobarbital sleep time. In these experiments, 24 hr after final drug treatment, sleep times for all treated groups were indistinguishable from their controls. Although the results were not included in Table 1, the sleep times for some of the CBD-treated groups (4, 8 and 12 days) were also normal 48 hr after withdrawal from CBD. The absence of a below-normal sleep time suggests that CBD does not induce the hepatic enzymes responsible for hexobarbital metabolism.

The pharmacokinetic data obtained support the hypothesis that the drug interaction property of CBD is due to one or more metabolites; however, the present study does not include any direct evidence for the presence of metabolic inhibitors. Such evidence has been described for the classical inhibitor of drug metabolism, SKF 525-A, by Buening and Franklin [15] who showed that the inhibition characteristically associated with this drug is probably partly dependent upon the formation of an inhibitory metabolite. Whether a similar situation obtains for CBD remains to be determined. A more detailed understanding of the mechanism by which CBD interferes with hepatic drug metabolism is important, not only because of the widespread use of marihuana, but also because CBD, as well as other

cannabinoids, is potentially a useful therapeutic agent [16].

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