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Influence of Fasting on the Absorption and Effects of Δ^9-Tetrahydrocannabinol after Oral Administration in Sesame Oil[1]

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PRYOR, G. T., S. HUSAIN AND C. MITOMA. *Influence of fasting on the absorption and effects of Δ^9-tetrahydrocannabinol after oral administration in sesame oil.* PHARMAC. BIOCHEM. BEHAV. 6(3) 331–341, 1977. – Tissue levels of ^{3}H were higher 2 hr after oral administration of ^{3}H-Δ^9-THC (10 mg/kg in sesame oil) to male Fischer rats in the morning compared with treatment in the afternoon. A corresponding reduction in potency was seen for the impairing effect of Δ^9-THC on performance of a conditioned avoidance response (CAR). The hypothesis that these effects were related to the interval between feeding (which normally occurs during the night in the nocturnal rat) and drug administration was supported when they were mimicked in overnight fasted and ad lib fed rats. Food deprivation decreased the rate of gastrointestinal absorption of ^{14}C-Δ^9-THC in sesame oil. Peak plasma levels of ^{14}C occurred 2–4 hr after administration in fed rats compared with 8 hr in fasted rats. When tested 2 hr after oral administration, Δ^9-THC caused significantly greater impairment of CAR performance in fed than fasted rats, whereas the opposite was found after 8 hr. Extraction and subsequent thin layer chromatography of plasma and brain from fed and fasted rats sacrificed 2 or 8 hr after oral administration of 10 mg/kg ^{14}C-Δ^9-THC showed that brain levels of 11-hydroxy-Δ^9-THC rather than Δ^9-THC were correlated with the behavioral effect.

Δ^9-THC 11-OH-Δ^9-THC Conditioned avoidance response Gastrointestinal absorption of Δ^9-THC
Plasma and tissue levels of Δ^9-THC Effects of food deprivation

WE HAVE been engaged in an extensive evaluation of the behavioral, pharmacological, and metabolic interactions between Δ^9-tetrahydrocannabinol (Δ^9-THC) and a number of other drugs in rats [15, 16, 17]. To avoid any possible direct chemical interactions between Δ^9-THC and the other drugs we administered each by a separate route. We used sesame oil as the vehicle for Δ^9-THC based on solubility characteristics of Δ^9-THC, ease of preparation, storage qualities, and the absence of any appreciable acute or subacute toxic or pharmacological effects of sesame oil alone [19]. Other solubilizers or dispersing agents such as ethanol, propylene glycol, or polysorbate were discounted because they have been reported to have pharmacological effects of their own (e.g. [3, 19, 21]) and the possibility of their interacting with Δ^9-THC and/or the other drugs was a complication we wished to avoid. Because we used sesame oil as the vehicle for Δ^9-THC and subacute treatment was involved, we also chose the oral route of administration. The other drugs were dissolved in an aqueous vehicle and administered IP.

During the course of this investigation we observed what appeared to be a major difference in the absorption and effects of Δ^9-THC when given in the morning or in the afternoon. These observations prompted the series of experiments described here, the results of which provide strong evidence that the digestive state of the rat at the time of oral administration of Δ^9-THC in sesame oil influences both the pharmacokinetics and pharmacodyamics of the compound in an unexpected way. Moreover, the results suggest that the pharmacologically active metabolite of Δ^9-THC, 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-Δ^9-THC), rather than Δ^9-THC itself, may have been responsible for the impairment in performance of a conditioned avoidance response (CAR).

EXPERIMENT 1

Effects of Subacute Treatment with Δ^9-THC on Tissue Levels of Δ^9-THC and Its Metabolites: Differences in Morning or Afternoon Administration of ^{3}H-Δ^9-THC

The data presented below were compiled from a series of 3 experiments designed to investigate the interactions between Δ^9-THC and other drugs. The data were analyzed with respect to the effects on tissue levels of ^{3}H of (a) subacute pretreatment for 6 days with sesame oil or unlabeled Δ^9-THC before administration of ^{3}H-Δ^9-THC

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and (b) administration of ^{3}H-Δ^9-THC in the morning or in the afternoon.

Method

Animals. Male rats of the inbred Fischer strain were used. They were 55 to 60 days old (140 to 160 g) when received from Simonsen Laboratories, Gilroy, California. They were housed singly in wire mesh hanging cages with food and water available at all times throughout the experiment. The ambient temperature was 22°C and the lights in the animal room were turned on at 0700 and off at 1900 daily.

Treatment and sacrifice. Separate groups of 15 rats each were intubated with 2 ml/kg sesame oil or 10 mg/kg Δ^9-THC (in sesame oil, 2 ml/kg) daily for 6 days. On the seventh day each animal was intubated with 10 mg/kg ^{3}H-Δ^9-THC (67.9 μCi/kg) and sacrificed 2 hr later. This time of sacrifice was chosen to coincide with the beginning of behavioral testing in parallel experiments and corresponded to the approximate time of peak effect by this route of administration in sesame oil [15, 16, 17]. The ^{3}H-Δ^9-THC was administered at 0835 (N = 9) or 1305 (N = 6) in the groups pretreated subacutely with sesame oil and at 0925 (N = 9) or 1355 (N = 6) in the groups pretreated subacutely with Δ^9-THC.

Procedure. Total radioactivity (representing unchanged Δ^9-THC plus any metabolites) was determined in plasma, brain, liver, kidney, lung, heart, perirenal fat, small intestine, and large intestine. Aliquots (0.1 ml) of plasma or a water homogenate (1:3, w/v) of each tissue were transferred to a counting vial. The homogenate of the gastrointestinal organs included their contents. Excessive coloration of each solution was removed by heating at 60°C for approximately 20 min after adding 3 or 4 drops of 30% hydrogen peroxide. After adding 10 ml of Scintisol-Complete (Isolab, Inc.) the radioactivity in each sample was quantitated using a Beckman LS-250 liquid scintillation system. Each sample was counted in duplicate. Correction for quenching was made with automatic external standardization and reference to a previously prepared quench curve. The cpm were converted to μg/ml or μg/g of Δ^9-THC equivalents in each tissue based on the specific activity of the administered ^{3}H-Δ^9-THC. The radioactivity in the gastrointestinal tract was expressed as a percentage of the administered radioactivity.

Data analysis. The data in this and subsequent experiments were first analyzed for statistical reliability using an appropriate analysis of variance [9]. Selected pairs of means were then compared by *t*-test using the pooled within group variance and associated degrees of freedom from the analysis of variance.

Results

The concentrations of ^{3}H in each tissue and for each group are shown in Fig. 1. The results of the analyses of variance computed for each tissue are summarized in Table 1. None of the 2-way interactions between replications and the other main effects were statistically significant. Therefore, the data for the three experiments were combined in Fig. 1.

In all tissues except perirenal fat ($p>0.05$) subacute pretreatment with 10 mg/kg/day Δ^9-THC for 6 days caused a significant increase in radioactivity derived from the ^{3}H-Δ^9-THC given 2 hr earlier on the seventh day compared with sesame oil pretreated controls (all $Fs\geq 10.4$ all $ps<0.01$). Also, the radioactivity was significantly higher in all tissues when the ^{3}H-Δ^9-THC was given in the morning compared with administration in the afternoon (all $Fs\geq 5.2$, all $ps\leq 0.05$). The interaction terms between pretreatment dosing and time of ^{3}H-Δ^9-THC administration were significant in plasma, brain, kidney, and heart and trends in this direction were evident in the other tissues. Comparisons between pairs of means showed that radioactivity was significantly higher after subacute pretreatment with Δ^9-THC than in sesame oil pretreated controls when ^{3}H-Δ^9-THC was administered in the morning

TABLE 1

F-RATIOS AND ASSOCIATED DEGREES OF FREEDOM FROM THREE-WAY ANALYSES OF VARIANCE OF RADIOACTIVITY IN PLASMA AND OTHER TISSUES

	Source of Variance						
Tissue	Reps. (R)	Pretreatment (P)	Time (T)	R x P	R x T	P x T	R x P x T
Plasma	8.6†	32.9†	29.4†	1.3	1.4	12.0*	1.0
Brain	2.6	14.3†	19.5†	0.0	1.7	4.7*	0.8
Liver	0.9	10.4†	6.7†	1.6	1.4	1.5	0.1
Kidney	0.7	20.9†	13.9†	1.2	0.5	5.9*	0.9
Lung	0.6	19.8†	12.3†	2.8	2.0	2.1	2.0
Heart	0.7	20.1†	15.3†	0.8	1.6	5.2*	0.4
Fat	5.9*	4.0	5.2*	1.2	0.0	1.4	1.3
Degrees of Freedom	2,18	1,18	1,18	2,18	2,18	1,18	2,18

*$p<0.05$.
†$p<0.01$.

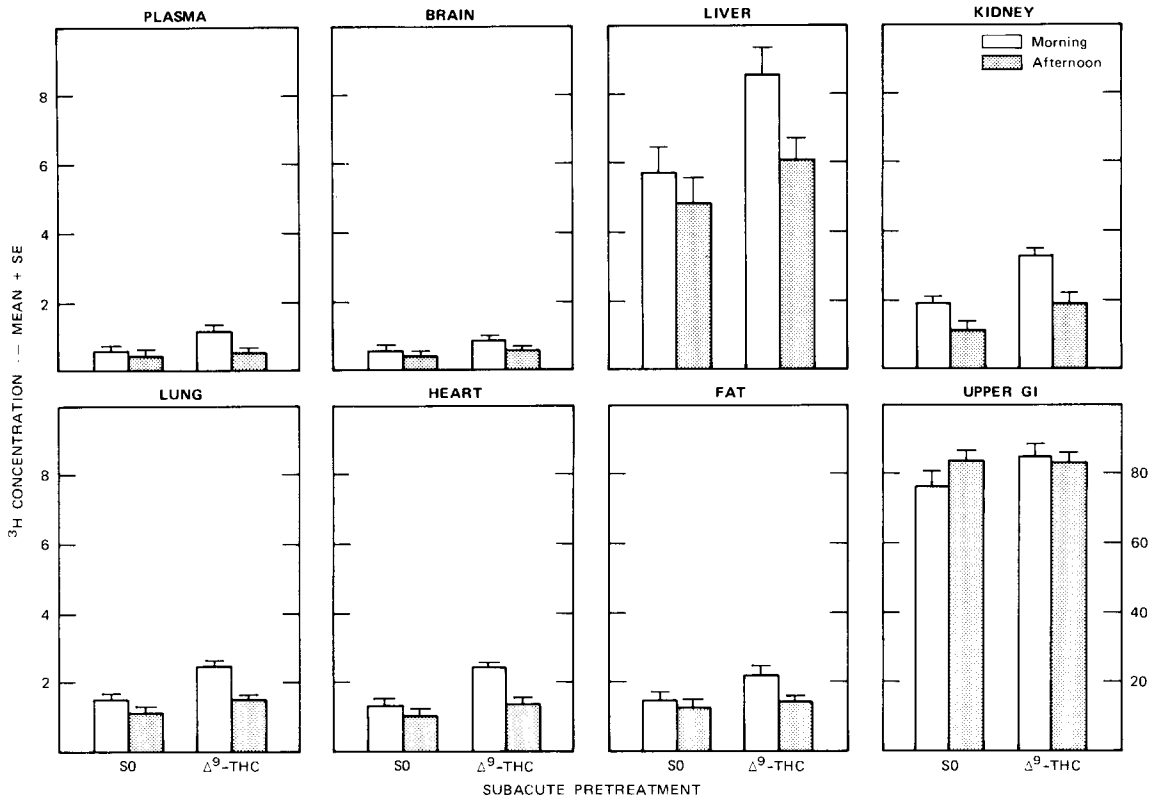

FIG. 1. Tissue distribution of radioactivity in rats intubated with 10 mg/kg ^{3}H-Δ^9-THC (67.9 μCi/kg) in the morning or afternoon and sacrificed 2 hr later. The rats were pretreated for 6 days with sesame oil (SO) or 10 mg/kg Δ^9-THC. Values for the Upper GI tract are expressed as a percentage of the administered radioactivity. Values for the other tissues are expressed as μg equivalent of Δ^9-THC per ml plasma or per g wet tissue.

(all $ts \geq 2.82$, all $ps \leq 0.05$). However, when ^{3}H-Δ^9-THC was given in the afternoon, the increase in radioactivity caused by subacute pretreatment with Δ^9-THC was only significant in kidney, $t = 2.7$, $p \leq 0.05$, although trends in this direction were evident in all other tissues. Conversely, the differences in radioactivity that were seen between morning and afternoon administration were most evident in the groups pretreated subacutely with Δ^9-THC (all $ts \geq 2.4$, all $ps \leq 0.05$); the difference was significant only in kidney ($t = 2.8$, $p < 0.05$) in the groups pretreated with sesame oil, although the same trends were evident in all other tissues.

The radioactivity remaining in the gastrointestinal tract was between 80 and 90% of the administered dose of ^{3}H-Δ^9-THC. There were no significant differences in residual radioactivity in the gastrointestinal tract among the groups that could account for the differences in the various tissues. However, even small differences in absorption that might have been obscured by the variability in the data might have resulted in large differences in tissue levels. Also, since Δ^9-THC is rapidly and extensively metabolized and its metabolites are excreted mainly via the bile [1,7], values for the gastrointestinal tract may not represent unabsorbed and unmetabolized ^{3}H-Δ^9-THC only.

EXPERIMENT 2

Effect of Fasting and Vehicle on Tissue Levels of Δ^9-THC and Its Metabolites

The results presented for Experiment 1 indicated that the tissue levels of radioactivity from ^{3}H-Δ^9-THC given orally in sesame oil were significantly influenced by the time of day when the drug was administered. Since the rat is nocturnal and does most of its feeding at night, the contents of the gastrointestinal tract at the time of oral administration of Δ^9-THC could influence its absorption and, therefore, the tissue levels attained. Thus, treatment in the afternoon would be expected to be on a relatively empty stomach compared with administration in the morning. The contents of the stomach are known to affect absorption of drugs by influencing gastric pH, rates of emptying, and intestinal motility [4]. Indeed, most drugs are absorbed better from an empty rather than a full stomach which leads to an expectation of results just the opposite of those found for Δ^9-THC in Experiment 1. However, this effect applies primarily to drugs in aqueous vehicles and food in the stomach accompanied by an active digestive state might actually facilitate absorption of Δ^9-THC from sesame oil. Therefore, we compared the plasma and tissue levels of radioactivity derived from ^{3}H-Δ^9-THC in animals fasted overnight with those in nonfasted animals when the ^{3}H-Δ^9-THC was given orally in sesame oil or in a Tween-80-saline formulation.

Method

The age, strain, and sex of the rats were the same as in Experiment 1. One group of 20 animals was treated daily in the morning for 6 days with 10 mg/kg Δ^9-THC suspended in 10% Tween-80 in saline. Another group of 18 animals was treated similarly with 10 mg/kg Δ^9-THC dissolved in

sesame oil. All treatments were given by intragastric intubation in 2 ml/kg of the vehicle. Food was removed from half of the animals in each group at 1600 on the sixth day. On the morning of the seventh day 10 mg/kg ^{3}H-Δ^{9}-THC was given orally in the respective vehicles (Tween-80, 80 μCi/kg; sesame oil, 227 μCi/kg) and the animals were sacrificed 2 hr later. Concentrations of ^{3}H were determined in plasma, brain, and liver by the methods described in Experiment 1.

Results

Fasting significantly affected the levels of radioactivity in all 3 tissues, but the direction of the effect depended on the vehicle used (Fig. 2). The interaction term of each analysis of variance was significant (all $Fs(1,31) \geq 15.6$, all $ps \leq 0.001$). When ^{3}H-Δ^{9}-THC was given in Tween-80-saline significantly higher plasma and tissue levels of radioactivity were found in fasted compared with fed animals, whereas the opposite was found when sesame oil was used as the vehicle. This latter result is the same as observed in Experiment 1 comparing morning and afternoon administration of ^{3}H-Δ^{9}-THC and suggests that the difference observed in that experiment could be accounted for in part, at least, by the digestive state of the animals at the time of treatment. The highest levels of radioactivity 2 hr after administration were achieved when ^{3}H-Δ^{9}-THC was given in Tween-80-saline to fasted animals and the lowest levels were achieved when ^{3}H-Δ^{9}-THC was given in sesame oil to fasted animals. Radioactivity remaining in the gastrointestinal tract did not reliably differentiate the various groups.

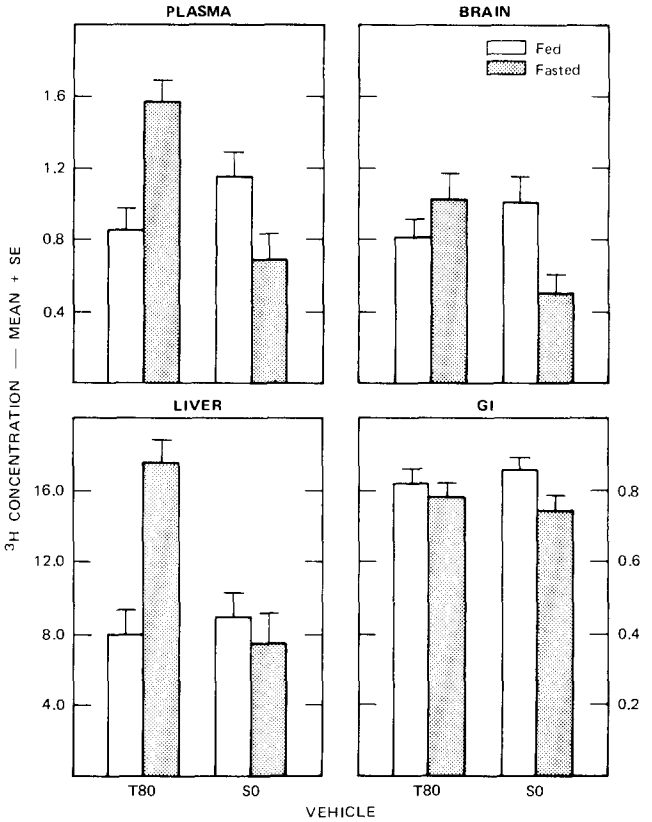

FIG. 2. Tissue concentrations of radioactivity in fed or fasted rats intubated with 10 mg/kg ^{3}H-Δ^{9}-THC in Tween-80-saline (80 μCi/kg) or sesame oil (227 μCi/kg) and sacrificed 2 hr later. The rats were pretreated for 6 days with 10 mg/kg Δ^{9}-THC in their respective vehicles. Values for the GI tract are expressed as a percentage of the administered radioactivity. Values for the other tissues are expressed as μg equivalents of Δ^{9}-THC per ml plasma or per g wet tissue.

EXPERIMENT 3

Time Course of Radioactivity in Plasma After Administration of ^{14}C-Δ^{9}-THC to Fed or Fasted Rats

The results of the first 2 experiments clearly established that higher levels of radioactivity were achieved 2 hr after oral administration of ^{3}H-Δ^{9}-THC in sesame oil to animals that had recently eaten compared with animals that were purposely fasted or were presumed to have been voluntarily without food for several hr. Thus, the absorption of Δ^{9}-THC in sesame oil as well as Tween-80-saline appeared to be influenced, albeit in opposite directions, by the digestive state of the animal at the time of drug administration. However, these results were obtained at only 1 time point after administration of ^{3}H-Δ^{9}-THC. To provide further evidence for this hypothesis, we compared the time course of plasma radioactivity derived from ^{14}C-Δ^{9}-THC administered orally in sesame oil to fed and fasted animals. Any difference in the rate of absorption would presumably be reflected in a difference in the times at which peak plasma levels of radioactivity were reached.

Method

Ten animals of the same age, strain, and sex as those in the first 2 experiments were used. Food was removed from 5 of the animals at 1600 on the day before the experiment. Beginning at 0830 the next morning, 10 mg/kg ^{14}C-Δ^{9}-THC (40 μCi/kg, 2 ml/kg) in sesame oil was given by intragastric intubation to each animal. Periocular blood was sampled repeatedly in each animal 1, 2, 4, 8, 12 and 24 hr later using 70 μl heparinized, disposable pipettes. After centrifugation, a constant, 30 mm section of each pipet containing 30 μl of the plasma was transferred to a counting vial containing 10 ml of Oxifluor-H_2O™ (New England Nuclear) for determination of radioactivity.

Results

The amounts of radioactivity in plasma increased to reach a peak between 2 and 4 hr after administration of ^{14}C-Δ^{9}-THC in the group of animals allowed free access to food (Fig. 3) and declined thereafter. In contrast, plasma levels of radioactivity in the fasted animals were lower during the first 4 hr than in the fed animals and did not reach a peak until about 8 hr. The peak levels of radioactivity achieved in both groups, although occurring at different times after administration, were about the same. The results of this experiment clearly support the hypothesis that fasting retards the absorption of Δ^{9}-THC in sesame oil from the digestive tract.

EXPERIMENT 4

Effect of Fasting, Vehicle, and Route of Administration on the Impairment of Avoidance Conditioning Caused by Δ^{9}-THC

The differences in tissue levels of radioactivity derived

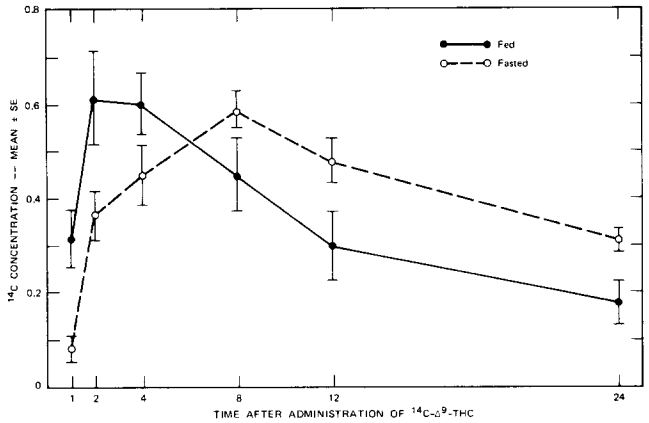

FIG. 3. Concentrations of radioactivity in the plasma of fed or fasted rats as a function of time after acute intubation of 10 mg/kg ^{14}C-Δ^9-THC (40 μCi/kg).

from ^{3}H-and ^{14}C-Δ^9-THC observed in the previous experiments are accompanied by differences in the pharmacological effect of Δ^9-THC as well. As in Experiment 1, retrospective analysis of data from other experiments in our laboratory showed that animals treated with 10 mg/kg Δ^9-THC in the afternoon or early evening performed a conditioned avoidance response (CAR) significantly better than comparable animals treated in the morning. Therefore, the purpose of this experiment was to determine whether or not the apparent diurnal effect of Δ^9-THC on CAR performance was also related to the digestive state at the time of drug administration and, therefore, correlated with tissue levels of radioactivity. In addition, we sought an alternative method of drug administration that might be unaffected by digestive state, since the logistics of our main investigation of the interactions between Δ^9-THC and other drugs [15,17] prohibited controlled feeding. Therefore, a parallel experiment was done in which the effect of Δ^9-THC dispersed in Tween-80-saline and administered IP was compared in fed and fasted animals.

Method

Animals. The 96 rats used in this experiment were of the same age, strain, and sex as those used in the previous experiments.

Apparatus. Each avoidance chamber consisted of a 30 × 36 × 40 cm wooden box housed inside a larger, sound-attenuated cabinet. Scrambled constant current 1.0 mA shock applied to 0.32-cm-diameter brass rods spaced 1.27 cm apart served as the unconditioned stimulus (UCS). Downward displacement (0.16 cm) of a 1.27-cm-diameter aluminum pole suspended from the center of the ceiling served as the conditioned response. A 7.5-W light and a 11.4-cm-diameter loudspeaker provided ambient light (0.44-ft-c measured at floor level) and an ambient 4-kHz tone (8 db above background, that was 50 db measured at the center of the floor using a General Radio Co. Type 1551-C sound-level meter set for A weighting). An increase in intensity above ambient, that occurred at the rate of 2.5 times per sec of either the light (to 0.88 ft-c) or the tone (to 63 db), or a low-intensity, nonaversive current (120 μA) that was applied to the floor served as conditioned stimuli (CS). Each chamber was ventilated and the fans contributed to the background noise. Twelve such chambers were interfaced with a Digital Equipment Corporation PDP 8/F computer (located in an adjoining room) that provided automatic control and data collection.

Procedure. The animals were trained in a single 30-trial session to escape footshock by pulling a 20-cm pole. Each trial lasted 30 sec unless the animal responded sooner. The intertrial interval was variable and averaged 60 sec (15 to 120 sec). Then they were given 3 daily 60-trial sessions to learn to avoid footshock by pulling a 13-cm pole in the presence of each of the 3 CS (tone, light, or nonaversive footshock), that preceded the UCS by 10 sec. The CS and UCS remained on for 30 sec, unless the trial was terminated earlier by a pole-displacement response. The 3 CS were presented randomly for 20 trials each. The entire 60-trial session required 2 to 2.5 hr. The intertrial interval was variable, averaging 1.5 min (15 to 180 sec). This schedule typically results in 80% avoidance or better to all 3 CS by the end of training.

After acquisition of the CAR, food was removed from 46 animals at 1600 the night before the test. The other 48 animals continued on ad libitum food access. On the next day 2 groups were given sesame oil (2 ml/kg) or 10 mg/kg Δ^9-THC in 2 ml/kg of sesame oil by intragastric intubation. The other 2 groups were given 10% Tween-80 in saline (2 ml/kg) or 10 mg/kg Δ^9-THC in 2 ml/kg of Tween-80-saline by IP injection. All animals were tested 2 hr after their respective acute treatments. The test session was conducted in the same way as the CAR training sessions.

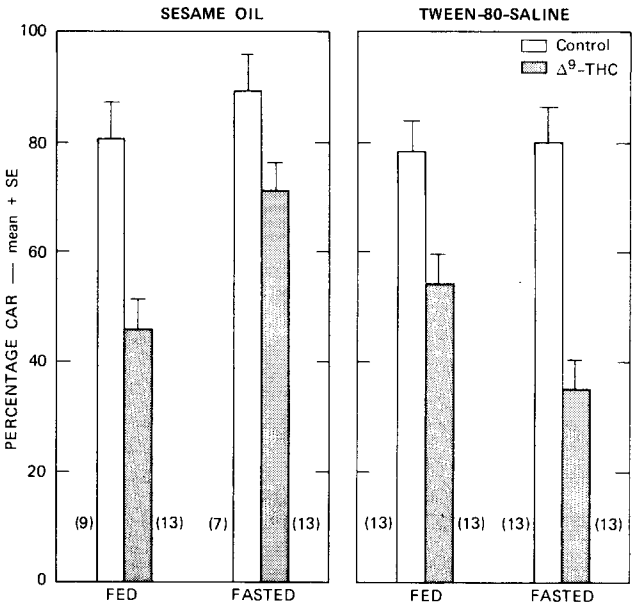

FIG. 4. Acute effects of 10 mg/kg Δ^9-THC on performance of a CAR when given orally in sesame oil or IP in Tween-80-saline to fed or fasted rats. Numbers of animals in each group are shown in parentheses.

Results

The results were essentially the same for all 3 stimuli used as CS and, therefore, the data were combined in Fig. 4 as percentage total CAR. The response to 10 mg/kg Δ^9-THC depended significantly on the digestive state of the animal at the time of treatment as well as the vehicle and

route of administration as reflected by the triple interaction term of the analysis of variance, $F(1,86) = 7.5$, $p<0.01$. There were no significant differences among the 4 control groups that received vehicle only. Administration of Δ^9-THC in either vehicle to fed animals caused significant impairment of CAR performance. As predicted from the results in Experiment 2, the impairing effect of Δ^9-THC in sesame oil was significantly less in fasted animals compared with fed animals, t (86) = 3.3, $p<0.01$. On the other hand, the effect of Δ^9-THC in Tween-80-saline was significantly greater, t (86) = 2.6, $p<0.01$, when given to fasted compared with fed animals.

The results of these experiments showed that the behavioral response to Δ^9-THC was influenced by the digestive state of the rat at the time of drug administration regardless of whether the drug was given orally or IP. The results of Experiments 2 and 3 provide an explanation of this effect in terms of differences in rates of gastrointestinal absorption after oral administration. Comparable data after IP administration in Tween-80-saline are not available to correlate the behavioral response with plasma or tissue levels of the drug or its metabolites. However, in view of the widespread physiological changes (e.g., nutrient levels, vascular distribution) that accompany feeding it is not unlikely that differences in absorption, distribution, etc. also occur after administration by this route. At any rate use of Tween-80-saline as a vehicle and administration by the IP route did not circumvent the influence of digestive state on the response to Δ^9-THC regardless of the mechanisms involved.

EXPERIMENT 5

Correlation of Behavioral Response with Plasma and Brain Levels of Δ^9-THC and Its Metabolites in Fasted Rats

If the differences in levels of radioactivity observed in plasma between fed and fasted animals are related to the pharmacological effects of Δ^9-THC, then we would have to predict from the results of Experiment 3 that such effects would be greatest around 2 to 4 hr in fed animals and around 8 hr in fasted animals. However, the levels of radioactivity in plasma may not accurately reflect the levels in brain. Furthermore, since radioactivity as measured in the previous experiments was undifferentiated and, thus, included unchanged Δ^9-THC and its metabolites, it is not possible to make this prediction with complete confidence, because the radioactivity in plasma at 8 hr could represent only inactive metabolites. Therefore, we compared the effects of Δ^9-THC on CAR performance in fed and fasted animals after 2 and 8 hr and, in parallel experiments, we examined the levels of Δ^9-THC and its metabolities in plasma and brain.

Method

Animals. The age, strain, and sex of the rats were the same as those in the previous experiments.

Behavior. The apparatus and procedures for establishing a CAR were the same as described in Experiment 4. After acquisition of the CAR food was removed from 24 animals at 1600 the night before the test. The other 24 animals continued on ad libitum food access. On the next day separate groups were given sesame oil (2 ml/kg, N = 16) or 10 mg/kg Δ^9-THC (N = 24) in sesame oil by intragastric intubation. Half of the animals in each group was tested for CAR performance 2 hr after acute treatment. The other half was tested 8 hr after acute treatment.

Biochemistry. Two experiments were conducted using 24 animals in each. The experiments were the same except that in the first, pooled extracts were analyzed by thin layer chromatography (TLC), whereas in the second the analysis by TLC was done on each sample. The animals were treated orally daily for 6 days with sesame oil. Food was removed from half of the animals at 1600 on the sixth day. Beginning at 0800 on the seventh day each animal in the first experiment was given 10 mg/kg ^{14}C-Δ^9-THC (40 μCi/kg) in sesame oil by intragastric intubation; the dose of radioactivity in the second experiment was 51 μCi/kg. Half of the animals were sacrificed by decapitation 2 hr and the other half 8 hr after acute drug administration. Blood was collected into heparinized tubes and the whole brain was rapidly removed, weighed, frozen on solid CO_2, and stored at $-20°$C for subsequent analysis.

After centrifugation of the blood, radioactivity was measured in 0.1 ml of the plasma. An aliquot (0.5 ml) of the remaining plasma was diluted with 0.5 ml distilled H_2O, and extracted twice with 5-ml portions of water-saturated ethyl acetate. The combined extracts were evaporated to 3 ml and the radioactivity in 0.1 ml of each extract was determined. In the first experiment the extracts were then pooled for each treatment group and time of sacrifice. In the second experiment each extract was treated separately. The pooled or separate extracts were evaporated to dryness under nitrogen and the residues were redissolved in 0.2 ml ethyl acetate. A 20-μl aliquot of each extract was spotted on a 10 × 20 silica gel precoated plate (Brinkmann). Authentic samples of ^{14}C-Δ^9-THC, ^{3}H-11-OH-Δ^9-THC, and 8,11-diOH-Δ^9-THC were also spotted in the middle of the plate to serve as standards in tentatively identifying the compounds present in the extract. The plates were developed using a chloroform:acetone (1:1, v/v) solvent system. The standards were visually located with ultraviolet light (254 nm). Bands corresponding to the migration of the 3 standards were scraped from the plates, eluted with 1 ml methanol, and the radioactivity in each was determined by liquid scintillation spectroscopy. The radioactivity in a fourth band with an Rf lower than that of 8,11-diOH-Δ^9-THC and that included the origin was also determined.

Whole brain was homogenized in 3 volumes of 0.9% saline (w/v) and 0.5 ml of the homogenate was diluted with 0.5 ml of distilled H_2O. The diluted homogenate was then extracted and either the pooled or separate extracts were further processed in the same way as plasma.

Results

Figure 5 shows the results for CAR performance. The interaction between drug treatment, deprivation, and time of testing was significant, $F(1,40) = 6.9$, $p<0.025$. There were no significant differences among the 4 control groups given sesame oil. When the animals were tested 2 hr after Δ^9-THC, the results were the same as in Experiment 4. That is, Δ^9-THC caused a significant impairment of performance relative to controls in the fed animals, $t(40) = 3.3$, $p<0.01$, but not in the fasted animals, $t(40) = 1.6$, $p>0.1$; the difference between the groups treated with Δ^9-THC was also significant, $t(40) = 2.3$, $p<0.05$. At 8 hr, the opposite results were found. The fed animals treated with Δ^9-THC were not significantly different from their controls, $t(40) = 0.8$, $p>0.1$, demonstrating recovery from

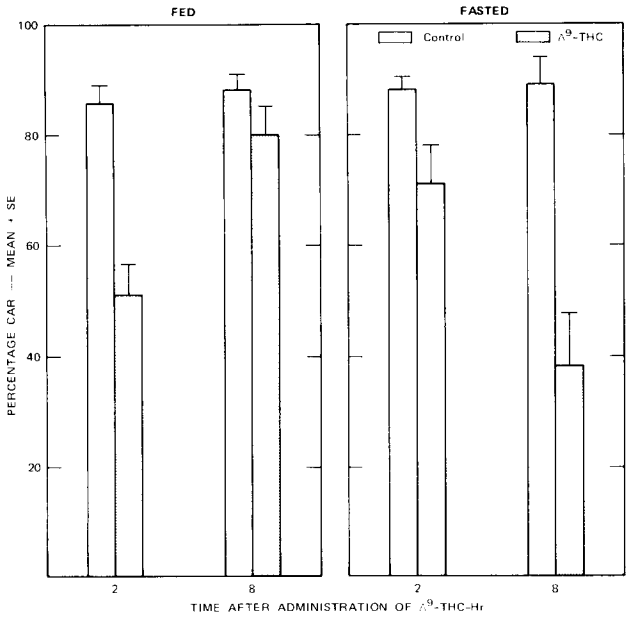

FIG. 5. Acute effects of 10 mg/kg Δ^9-THC on performance of a CAR when given orally in sesame oil to fed or fasted rats tested 2 or 8 hr after treatment. There were 4 rats in each control group treated with sesame oil and 8 rats in each group treated with Δ^9-THC.

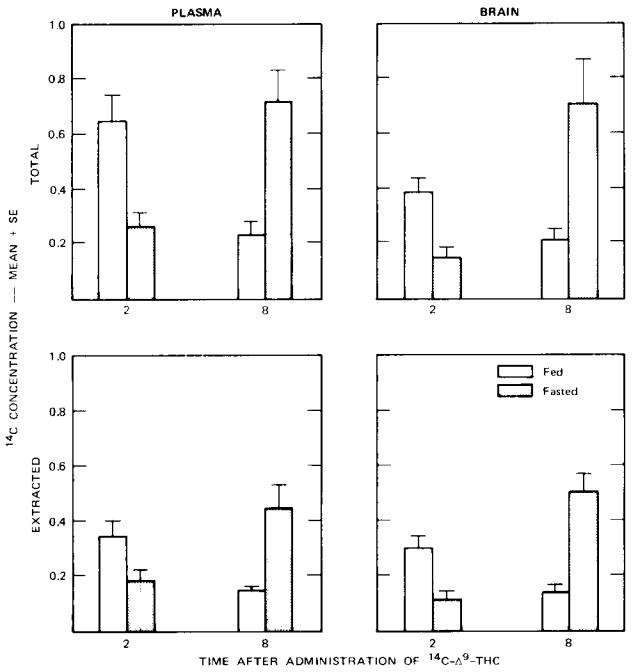

FIG. 6. Concentrations of total and ethyl acetate-extractable radioactivity in the plasma and brains of fed or fasted rats intubated with 10 mg/kg ^{14}C-Δ^9-THC (40 μCi/kg) and sacrificed 2 or 8 hr later. Values are expressed as μg equivalents of Δ^9-THC per ml plasma or per g wet tissue.

the acute effects of the drug, whereas performance was significantly impaired in the fasted animals treated with Δ^9-THC, $t(40) = 4.4$, $p<0.01$. Although there was a trend toward greater impairment in the fasted animals treated with Δ^9-THC at 8 hr than in the fed animals treated with Δ^9-THC at 2 hr, the difference was not significant, $t(40) = 1.5$, $p>0.1$.

Figure 6 shows the levels of radioactivity derived from ^{14}C-Δ^9-THC in plasma and brain along with the radioactivity that was extracted with ethyl acetate. In agreement with the results of Experiment 3, the levels of radioactivity in plasma were higher in fed than in fasted animals after 2 hr, $t(20) = 2.6$, $p<0.05$, whereas the reverse was true after 8 hr, $t(20) = 4.5$, $p<0.01$. In the fed animals the decrease in radioactivity between 2 and 8 hr was significant, $t(20) = 2.9$, $p<0.01$, as was the increase in the fasted animals, $t(20) = 4.2$, $p<0.01$. The same results were found in brain. Thus, the levels of total radioactivity in both plasma and brain were correlated with the behavioral effect of Δ^9-THC seen in the parallel experiment.

The radioactivity that was extracted with ethyl acetate paralleled the levels of total radioactivity and, therefore, they were also correlated with the behavioral effects; in every statistical comparison between fed and fasted animals the results for extracted radioactivity were equivalent to those for total radioactivity.

The percentage of radioactivity that was extracted was significantly less after 8 hr than after 2 hr in both plasma, $F(1,20) = 16.0$, $p<0.001$, and brain, $F(1,20) = 19.4$, $p<0.001$, indicating an increase in polar metabolites of Δ^9-THC at this later time. However, these differences in the percentage of extractable radioactivity were unrelated to the behavioral effects.

Inspection of the results of the TLC separation of the radioactivity from the pooled extracts suggested that the brain levels of apparent 11-OH-Δ^9-THC, rather than Δ^9-THC, may have been responsible for the effects on CAR performance. Levels of unchanged Δ^9-THC in brain appeared to be lower after 2 hr in both fed and fasted animals than they were after 8 hr, especially in the fasted animals. On the other hand, levels of 11-OH-Δ^9-THC in brain were perfectly correlated with the behavioral effect.

Because these preliminary results based on only single pooled extracts represented a potentially important finding, we repeated the experiment except that the TLC was done on each sample separately to provide a basis for statistical comparisons.

Figure 7 shows the results for total radioactivity and the radioactivity that was extracted with ethyl acetate in the repeat experiment. The similarity between these and the original results are clear and demonstrate the reproducibility of this effect. Statistical analysis of these data corroborated in all essential respects the conclusions reached and discussed above for the original experiment.

Figure 8 shows the results of separating the radioactivity by TLC. Again, the results of this experiment done with individual samples corresponded in all respects to the results obtained in the original experiment using pooled samples. Analysis of variance showed that the variations in apparent unchanged Δ^9-THC levels in plasma were not statistically reliable ($p>0.10$). The apparent high levels in the fed animals at 2 hr was caused by a single exceptionally high value and accounts for the large variability in this group. In brain the interaction term of the analysis of variance for levels of apparent unchanged Δ^9-THC was

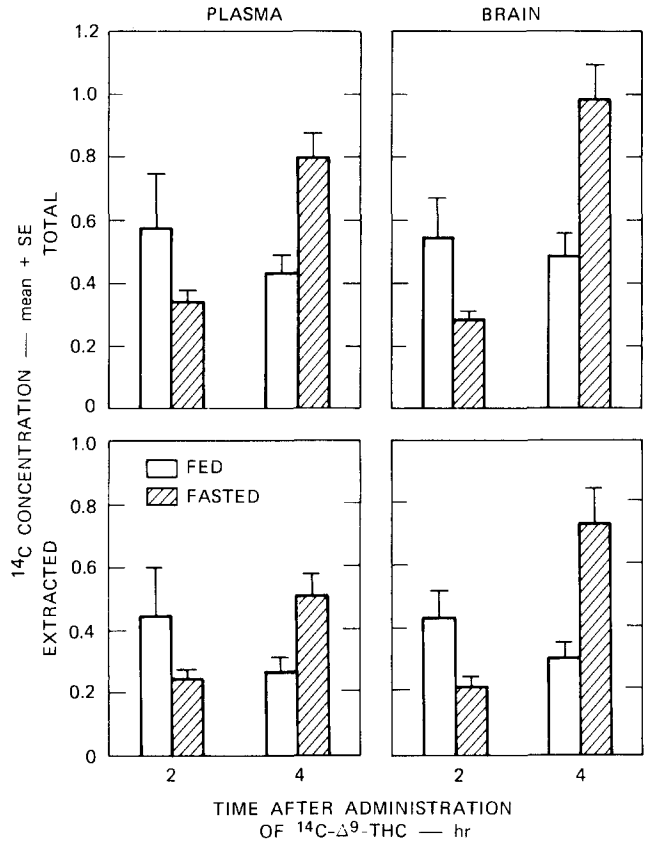

FIG. 7. Same as in Fig. 6. The experiment was repeated to verify the results and provide separate samples for tentative identification of unchanged Δ^9-THC and its metabolites.

significant, $F(1,20) = 14.7$, $p<0.001$. Individual comparisons showed that the levels of apparent unchanged Δ^9-THC in the fasted animals after 8 hr were significantly higher than the levels in any of the other groups (all ts $(20)>4.5$, all ps<0.01, two tailed). None of the differences among the other three groups were significant indicating that levels of apparent unchanged Δ^9-THC in brain were not significantly related to the behavioral effect.

On the other hand, the levels of apparent 11-OH-Δ^9-THC in both plasma and brain were related to the behavioral effect and the differences were statistically significant, $F(1,20) = 8.9$, $p<0.01$; $F(1,20) = 14.3$, $p<0.005$, for the interaction terms of the analyses of variance of plasma and brain, respectively. In brain at 2 hr the levels of 11-OH-Δ^9-THC were lower in fasted than in fed animals, $t(20) = 1.9$, $p<0.05$, one-tailed, whereas the reverse was found at 8 hr, $t(20) = 3.4$, $p<0.005$, one-tailed.

There was also a trend for the levels of apparent 8,11-diOH-Δ^9-THC in brain to be related to the behavioral effect, $F(1,20) = 7.0$, $p<0.025$, but only the difference at 2 hr was significant, $t(20) = 3.1$, $p<0.05$, one-tailed. Since the evidence suggests that this metabolite is pharmacologically inactive, it is unlikely that it was responsible for the behavioral effect. Instead, its correlation with the behavioral effect was probably the result of its association with 11-OH-Δ^9-THC as a secondary metabolite of Δ^9-THC. Similarly, the unidentified material on the plates was related to the behavioral effect in both plasma, $F(1,20) = 7.6$, $p<0.025$, and brain, $F(1,20) = 13.1$, $p<0.005$. We assume that the radioactivity associated with this region also represents inactive metabolites of Δ^9-THC more polar than 8,11-diOH-Δ^9-THC.

DISCUSSION

The results of these experiments have clearly demonstrated that the absorption and pharmacological effects of Δ^9-THC dissolved in sesame oil and administered intragrastically are enhanced by an active or recently active digestive system in the rat. In contrast most orally-administered drugs are absorbed better from an empty stomach. Two major factors account for the more general effect [4]. First, since most drugs are absorbed primarily from the intestine, any event that retards stomach emptying such as recent feeding, delays access of the drug to the absorption sites. Second, in the absence of food, the drug simply has greater access to the absorption surface. However, the use of sesame oil as a vehicle for the lipophilic Δ^9-THC may represent a special situation. In order to be absorbed effeciently, the sesame oil containing Δ^9-THC must intermix with the aqueous contents of the gastrointestinal tract and come in contact with the absorption surface. We suggest that food may act to disperse the sesame oil bolus and thus increase contact of Δ^9-THC with absorption sites of the intestine. Moreover, digestive enzymes and bile are increased in the intestine when food is present and thus absorption of Δ^9-THC may be further facilitated.

This interpretation can adequately account for the results obtained using the oral route of administration. However, in Experiment 4, we also found that the impairment of CAR performance caused by Δ^9-THC suspended in Tween-80-saline and injected intraperitoneally was greater in fasted than in fed rats. This result suggests that the digestive state of the rat may also influence the disposition and/or effects of Δ^9-THC when given by routes other than oral. Such an effect can also be rationalized on the basis of the known physiology of digestion. In addition to the digestive process that is activated by food ingestion, there is a redistribution of blood flow to the abdominal viscera and away from other areas including the brain. Thus, a change in the tissue distribution as well as the absorption of Δ^9-THC might occur. Also, since a major route of excretion of metabolized Δ^9-THC is through the bile into the intestine, the state of digestion can be expected to influence these processes as well.

Our results in the rat are somewhat at variance with those reported by Perez-Reyes *et al.* [14] in humans in terms of the efficiency of absorption from different vehicles. They had their subjects ingest capsules containing 35 mg ^{3}H-Δ^9-THC dissolved or suspended in five vehicles after a 12 hr fasting period. Measurement of ^{3}H in plasma indicated that the highest levels were achieved when sodium glycocholate was the vehicle, followed in order by sesame oil, Tween-80, ethanol and sodium glycocholate plus ethanol. These plasma levels were also correlated with the subjective high reported by the subjects. On the contrary, we found in Experiment 2 that higher levels of ^{3}H in plasma, brain, and liver were achieved when Δ^9-THC was administered orally 2 hr earlier to fasted rats in Tween-80-saline compared with sesame oil. Our results agree with those of Mantilla-Plata and Harbison [11] who reported that in mice higher plasma levels of radioactivity derived from ^{14}C-Δ^9-THC were achieved when given in Tween-80-

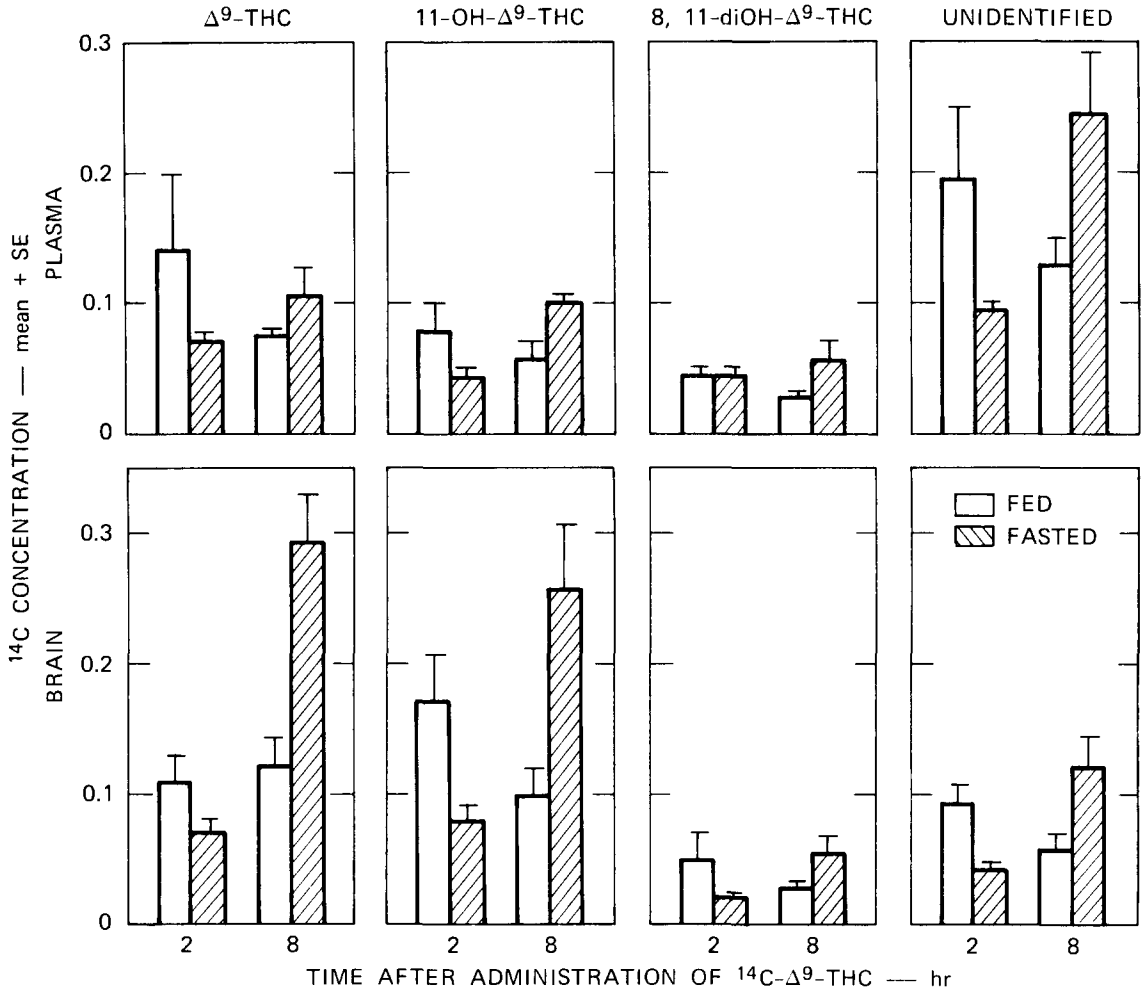

FIG. 8. Tentative identification of radioactivity associated with ethyl acetate extracts of plasma and brain from fed or fasted rats intubated with 10 mg/kg ^{14}C-Δ^9-THC (40 μCi/kg) and sacrificed 2 or 8 hr later. Values are expressed as μg equivalents of Δ^9-THC per ml plasma or per g wet tissue.

saline compared with corn oil. They also found that the toxic effects of Δ^9-THC were greater when administered IP in Tween-80-saline compared with corn oil [10]. Likewise, Rosenkrantz *et al.* [18] found a similar relationship in terms of the LD50 for Δ^9-THC in Fischer rats when the drug was given orally in sesame oil or as an emulsion in 7% sesame oil and 0.5% Tween-80. Thus, the data for rodents appear consistent but at variance with those for humans.

The results of Experiment 3 suggested that fasting caused a decrease in the rate of gastrointestinal absorption of Δ^9-THC rather than a reduction in the maximum levels achieved. Thus, the highest plasma levels of radioactivity were reached between 2 to 4 hr after administration in fed rats, whereas they were delayed to about 8 hr in fasted rats. These results prompted the interesting prediction that the time of peak effect on CAR performance would occur around 8 hr after treatment in fasted animals, whereas the time of peak effect would be at 2 to 4 hr in fed animals with recovery occurring by 8 hr. These predictions were realized in Experiment 5. Thus, the levels of radioactivity in plasma were correlated with one of the pharmacological effects of the drug. Moreover, this effect was found in Experiment 5 to be related to the levels of apparent 11-OH-Δ^9-THC in brain rather than with the levels of the parent compound. Mechoulam [16] proposed that 11-OH-Δ^9-THC may be solely responsible for the pharmacological effects of Δ^9-THC, although this issue has not been settled and conflicting data exist. Christensen *et al.* [2] found 11-OH-Δ^9-THC to be 18 times as potent at Δ^9-THC when administered intraventricularly. Since Δ^9-THC is not metabolized in brain they concluded that the effect of systemic administration was due to the metabolite. However, Gill and Jones [5] reported that levels of both Δ^9-THC and 11-OH-Δ^9-THC in brain were highly correlated with the cateleptic effect of Δ^9-THC in mice after IV administration. Later, Gill and Lawrence [6] also injected Δ^9-THC and 11-OH-Δ^9-THC directly into the cerebral ventricles and found them both to have the same activity with 11-OH-Δ^9-THC being only about twice as potent as Δ^9-THC. Sofia and Barry [20] reported that inhibition of hepatic microsomal drug metabolism with SKF 525A increased the ability of Δ^9-THC to prolong barbiturate sleeping time. They assumed that the metabolism of Δ^9-THC itself was the critical step involved and concluded that the parent compound was responsible for the pharmacological effect. However, this assumption was questioned by Gill and Jones

[5], who found that pretreatment with SKF 525A increased brain concentrations of Δ^9-THC by only about 23% compared with a threefold increase in 11-OH-Δ^9-THC. Thus, SKF 525A apparently inhibits the second step in the metabolism of Δ^9-THC as well as the first and can, under appropriate kinetics, result in lower levels of the parent compound than its metabolite. Therefore, the results of Sofia and Barry [20] do not prove that Δ^9-THC rather than 11-OH-Δ^9-THC was responsible for the pharmacological effect. However, neither do the other experiments prove that it was not. Our results are pertinent to this issue and appear to dissociate the effects of Δ^9-THC and 11-OH-Δ^9-THC at least on performance of a CAR in rats. Thus, if brain levels of unchanged Δ^9-THC were responsible for the behavioral effect, a threefold increase in potency should have occurred in the fasted animals 8 hr after treatment compared with the fed animals after 2 hr. Instead, there was no significant difference in effect in these 2 groups. On the other hand, brain levels of 11-OH-Δ^9-THC were directly related to the impairment caused by Δ^9-THC. However, if 11-OH-Δ^9-THC, in fact, was responsible for impairing CAR performance this does not necessarily imply that 11-OH-Δ^9-THC is solely responsible for all the diverse pharmacological effects of Δ^9-THC. Indeed, we have found in other experiments [unpublished] that the hypothermic and bradycardic effects of oral doses of Δ^9-THC in sesame oil ranging from 2.5 to 10.0 mg/kg were the same in fed and fasted rats, whereas results similar to those found for CAR performance were obtained for rotorod performance. Thus, whole brain levels of 11-OH-Δ^9-THC were uncorrelated with at least 2 pharmacological effects of Δ^9-THC. However, these effects were uncorrelated with levels of the parent compound as well. Clearly, we are still a long way from explaining the mechanisms involved in the effects of Δ^9-THC. Investigation of the distribution of Δ^9-THC and its metabolities within different regions or subcellular fractions of the brain as well as the binding of Δ^9-THC and/or its metabolites to cellular constituents may help elucidate the mechanisms involved.

The initial observation of differences in tissue levels and the effect of Δ^9-THC when administered in the morning or in the afternoon deserves further comment. We tentatively rejected the explanation for this effect as being a manifestation of diurnal variations in absorption, distribution, metabolism, elimination, or receptor sensitivity in favor of the gastrointestinal dynamics hypothesis because of the results of the subsequent experiments described herein. However, although gastrointestinal dynamics can adequately account for these observations, the possibility of a diurnal contribution to this effect cannot be summarily discounted on the basis of this evidence alone. Diurnal variations in the potencies of many drugs and their metabolism by hepatic enzymes have been well documented (see [13] for review). Similar results for Δ^9-THC have not as yet appeared, but it would not be surprising to find diurnal variations in the effects of this drug as well. Indeed, such variations, together with the effects we found for gastrointestinal activity, may help explain some of the discrepancies in the literature regarding the potency and metabolism of Δ^9-THC. In any event these effects should be recognized as important methodological parameters and their influence assessed or controlled accordingly.

Another result of Experiment 1 also requires comment. Plasma and tissue levels of ^{3}H were higher in rats that had been pretreated with 10 mg/kg Δ^9-THC for 6 days than in controls pretreated with sesame oil. This result has been repeated many times in our laboratory using both ^{3}H-Δ^9-THC and ^{14}C-Δ^9-THC [15, 16, 17]. A possible explanation for this result is that there is formed in each tissue of the pretreated rats, a Δ^9-THC-metabolites pool [8] that is displaced with the newly-introduced labeled Δ^9-THC-metabolites resulting in larger quantities of the labeled compounds being retained than in tissues of sesame oil-pretreated (control) rats. Whether or not this effect can in any way help explain the tolerance that develops to Δ^9-THC cannot be answered, however.

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