(98 percent) and analyzed for the remaining radioisotope. A summation of the radioactivity found in the medium and in the bone insured that pairs of bones had comparable total radioactivity (*6*). The inhibitory effect of 2-TCA on ^{45}Ca release from experimental half calvaria was calculated as the percentage of ^{45}Ca release inhibited by means of the formula $[(C - T)/C] \times 100$, where C represents ^{45}Ca in medium from the control half calvarium and T represents counts in medium from the half calvarium treated with 2-TCA. It has been demonstrated (*6*) that approximately 35 ± 5 percent of the total release of ^{45}Ca in this system is due to physicochemical exchange between bone and medium and is unaffected by hormones and vitamins. Therefore, the maximum inhibitory effect on a metabolically mediated process can be only 65 ± 5 percent of the total release of ^{45}Ca from the control bones. The results in Table 1 show that at doses as low as 5 to 10 μg/ml, 2-TCA significantly inhibited the cell-mediated ^{45}Ca transfer out of labeled bone.

To study further the inhibitory effect of 2-TCA on mineral loss from bone in culture system B, we cultured unlabeled half calvaria separately with or without 2-TCA. At the end of a 3-day incubation, they were stained by the Von Kossa reaction. The results (Fig. 2B) provide further evidence that 2-TCA inhibited directly the spontaneous resorption of bone in tissue culture.

Microscopic observations of the living culture and histological examination of bone sections showed that 2-TCA, at the dosages and time intervals used here, did not produce any cytotoxic effects, as judged by fibroblastic outgrowth and bone cell morphology.

The hypocalcemic effects of 2-TCA are very similar to those produced by the polypeptide hormone calcitonin (*2, 3*). Our findings demonstrate further the close similarity between the direct actions of 2-TCA and calcitonin on bone in tissue culture (*7*). However, 2-TCA is a simple compound and, therefore, may provide a useful, new pharmacological tool for studies of bone metabolism and calcium transfer.

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8. Supported in part by PHS grants AM-10206 and DE-02849. We thank Dr. A. H. Tashjian, Jr., for his critical advice and A. B. Flaminiano and Joan Jennings for their technical assistance.

9 November 1970

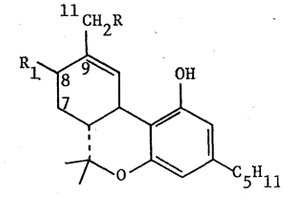
Activity of Δ^8- and Δ^9-Tetrahydrocannabinol and Related Compounds in the Mouse

Abstract. *The 11-hydroxy metabolites of* Δ^8- *and* Δ^9-*tetrahydrocannabinol are more active than the parent compounds when administered to mice by either the intravenous or intracerebral route. Both* Δ^8- *and* Δ^9-*tetrahydrocannabinol are rapidly and extensively metabolized by the liver and not by the brain. The hypothesis that the 11-hydroxy metabolites may be the active form of tetrahydrocannabinol is discussed.*

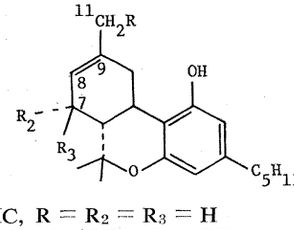
Δ^9-Tetrahydrocannabinol (Δ^9THC) is considered responsible for most of the psychotomimetic effects of hashish and marihuana (*1–3*). *trans*-Δ^9-Tetrahydrocannabinol (Δ^9THC) is the major natural THC, although small amounts of Δ^8THC also occur. The isolation and structural elucidation of two metabolites of Δ^9THC, 11-hydroxy-Δ^9THC and 8,11-dihydroxy-Δ^9THC, have recently been reported (*4*). The former metabolite has also been independently isolated (*5*). Others have reported on the isolation of 11-hydroxy-Δ^8THC (*2, 6*). These findings have been confirmed and the isolation of two additional metabolites of Δ^8THC, 7α- and 7β,11-dihydroxy-Δ^8THC has been reported (*7*). The structures of these compounds are shown below.

The biological activity of the above compounds plus other available cannabinoids, including cannabidiol (CBD), cannabinol (CBN), and $\Delta^{9(11)}$THC (*8*) was compared to that of Δ^9THC in the mouse after intracerebral and intravenous administration (*9*). Although the potency of the compounds varied depending on structure and route of administration (Table 1), similar neurological and behavioral responses were found for all of the substances provided that high concentrations of the less potent compounds were used.

The general overt behavioral pattern is characterized by three progressive stages: (i) irritability; (ii) decrease in spontaneous activity with hypersensitivity to auditory and tactile stimuli; (iii) marked depression of spontaneous activity, decreased awareness, loss of muscle coordination, reduced sensorimotor responses, and a crouched posture and gait similar to that produced by the narcotic analgesics (*10*). Provoked freezing or immobility, a property which is also induced by the narcotic analgesics, was much more pronounced with the Δ^8THC compounds. Hypothermia and decreased respiration rate also occur, and then death by respiratory arrest. When 11-hydroxy-Δ^9THC was administered intracerebrally, measurable behavioral alterations were observed at doses of



Δ^9THC, R = R$_1$ = H
11-hydroxy-Δ^9THC, R = OH, R$_1$ = H
8,11-dihydroxy-Δ^9THC, R = R$_1$ = OH



Δ^8THC, R = R$_2$ = R$_3$ = H
11-hydroxy-Δ^8THC, R = OH;
R$_2$ = R$_3$ = H
7α,11-dihydroxy-Δ^8THC, R = R$_2$ = OH;
R$_3$ = H
7β,11-dihydroxy-Δ^8THC, R = OH;
R$_2$ = H; R$_3$ = OH

Table 1. Relative potency of Δ^9THC to related compounds. The relative potency by either route is defined as the ratio of the number of milligrams of Δ^9THC administered intravenously to the number of milligrams of test compound (administered intravenously or intracerebrally) required to produce a significant decrease in spontaneous activity and hypersensitivity to tactile and auditory stimuli. Similar relative potencies were found when other indices such as marked depression, a significant change in one of the sensorimotor responses, or the crouched posture and gait were selected. The dosage necessary to cause respiratory arrest is not in all cases comparable to the above potencies.

Compound	Route of administration	
	Intravenous	Intracerebral
Δ^9THC	1.0	0.8
11-Hydroxy-Δ^9THC	2.0	15
8,11-Dihydroxy-Δ^9THC	>0.02	0.6
Δ^8THC	0.9	0.8
11-Hydroxy-Δ^8THC	2.0	14
7α,11-Dihydroxy-Δ^8THC	~0.04	~0.3
7β,11-Dihydroxy-Δ^8THC	~0.06	~0.7
$\Delta^{9(11)}$THC	0.05	0.7
Cannabidiol	0.03	0.5
Cannabinol	0.03	0.7

0.002 mg, and respiratory arrest occurred at doses of about 0.05 mg, as compared with doses of 0.05 and 0.5 mg, respectively, for Δ^9THC.

Table 1 shows four important features. (i) In the mouse by either route of administration Δ^8- and Δ^9THC are approximately equipotent; this holds also for their corresponding 11-hydroxy metabolites. (ii) By the intravenous route the 11-hydroxy metabolites are about twice as potent as the parent compounds and by intracerebral administration about 18 times as potent. (iii) The route of administration greatly affects the potency of the 11-hydroxy metabolites, which are approximately seven times more potent by intracerebral administration than by intravenous administration. In contrast, the parent compounds are approximately equipotent by either method of administration. (iv) Compounds such as cannabinol and cannabidiol, which by other routes of administration are considered inactive or at best very weakly active, are at least as potent as Δ^8- and Δ^9THC by intracerebral administration. The sequence of behavioral changes with increased concentration, however, was not always the same in the two groups of compounds.

In addition, both Δ^8- and Δ^9THC had a faster onset of activity after intravenous than after intracerebral administration, whereas with the 11-hydroxy metabolites the onset was slightly faster than with their parent compounds. Peak behavioral effects for all four compounds occurred within 10 to 15 minutes by both routes. The duration of overt changes was shorter after intracerebral than after intravenous administration. When 0.2 mg of Δ^9THC was administered intravenously the onset of activity occurred within 2 minutes; maximum responses were observed at approximately 10 minutes and lasted up to 4 hours, as compared to 3 minutes, 10 minutes, and 1 hour, respectively.

The foregoing data suggest that the activity after intravenous administration of Δ^8- or Δ^9THC may be due to conversion to the corresponding 11-hydroxy metabolite (*1*, p. 1165). To test this possibility, a study in vitro was conducted to see if Δ^9THC could be metabolized in certain organs of the mouse, including the brain.

Mouse liver, brain, small intestine, spleen, and red blood cells were homogenized and the fractions containing the microsomes and soluble enzymes were incubated with 1.0 mg of ^{14}C-labeled Δ^9THC (0.8 μc/mg) per gram of tissue for 1 hour in a 0.1*M* potassium phosphate buffer, *p*H 7.4, containing a reduced nicotinamide adenine dinucleotide phosphate (NADPH) regenerating system. The metabolites were separated by thin-layer chromatography, and the individual labeled peaks (*10, 11*) were isolated. A liquid scintillation counter was used to determine the yields. Under these conditions Δ^9THC was extensively metabolized in the liver (about 80 percent), and less so in the spleen (9 percent) and blood (14 percent); there was negligible conversion in the brain and small intestine (*12*).

If Δ^9THC activity is due to transformation to the 11-hydroxy metabolite, then the concentration of the metabolite in blood plasma should follow a time course similar to that of the overt activity. Proof of this assumption was obtained by administering Δ^9THC intravenously and intracerebrally and taking blood samples at 0.5, 3, 10, and 30 minutes (*13*). At 0.5 minutes after intravenous administration of Δ^9THC, the metabolite 11-hydroxy-Δ^9THC was demonstrated both by thin-layer and gas-liquid chromatography. Within 3 minutes both 11-hydroxy- and 8,11-dihydroxy-Δ^9THC were present after intravenous or intracerebral administration. The metabolite concentration reached a maximum 10 minutes after the drug was administered by either route, a time that approximately corresponded to the time of maximum behavioral effects (Table 2).

A comparison of the relative proportions of Δ^9THC to its total metabolites in specific organs of the mouse 10 minutes after administration (Table 2) showed that in blood, spleen, and kidney the ratios of Δ^9THC to metabolite were approximately 60 to 40. By both routes of administration the liver had metabolized more than 90 percent of the Δ^9THC. In the brain the ratio of Δ^9THC to metabolites was 70 to 30 after intravenous and 90 to 10 after intracerebral administration. By both routes of administration, 11-hydroxy and 8,11-dihydroxy-Δ^9THC accounted for more than 70 percent of the metabolites. The ratios of 11-hydroxy to 8,11-dihydroxy-Δ^9THC after intravenous administration were: blood, 60 to 40; liver, 70 to 30; and brain, 80 to 20. The same ratios were found when Δ^8THC was similarly administered.

Our data demonstrate that the 11-hydroxy metabolites of Δ^8- and Δ^9THC are much more potent when given intracerebrally than the Δ^8- or Δ^9THC compounds. The parent compounds are rapidly and extensively metabolized in the liver when given by either route. The Δ^9THC and its metabolites can pass the blood-brain barrier,

Table 2. Percentage recovery of ^{14}C-labeled Δ^9THC and metabolites 10 minutes after intracerebral or intravenous injection.

	Intracerebral (%)			Intravenous (%)		
	Total label	Δ^9 THC	Metabolites*	Total label	Δ^9 THC	Metabolites*
Brain	5.2	4.6	0.6	0.9	0.6	0.3
Blood (1 ml)	1.3	0.8	0.5	0.7	0.4	0.3
Liver	15.4	0.6	14.8	31.1	2.2	28.9
Spleen	0.2	0.12	0.08	0.3	0.17	0.13
Kidney	2.4	1.6	0.8	1.8	1.2	0.6
Injection site	10.8					
Total recovered	35.3			34.8		

*11-Hydroxy-Δ^9THC in each case was the major metabolite.

although the compounds may certainly vary in the proportions which pass from blood to brain and vice versa. Although the amount of metabolite in the brain after administration of Δ^8- or Δ^9THC by either route is small, there is enough to account for the biological activity, in view of the high potency of the 11-hydroxy metabolites. Therefore, the 11-hydroxy metabolites may be the active forms of THC in the mouse.

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9. Male CF-1 mice were used in our study. The profile of activity was determined according to S. Irwin [*Psychopharmacology* **13**, 222 (1968)]. The drugs were prepared as a suspension of 5 percent Tween 80 and water. For intracerebral administration the volume was 30 μl for each dose used; for intravenous administration, the dose was 0.1 ml per 10 grams of body weight. The intracerebral injection technique was that of T. J. Haley and W. C. McCormick [*Brit. J. Pharmacol.* **12**, 12 (1957)]. In general a minimum of three active dosages, with six animals at each, was used. A base-line dose response relation for Δ^9THC was established at 0.1, 1.0, 2.5, 5.0, 10.0, 25.0 mg/kg. The dose response relationship was established by criteria shown in Table 1. Metabolites were compared on a relative dose basis in a similar manner. The supplies of dihydroxy metabolites were too low to obtain full dose-response curves.
10. Similar behavioral changes have been reported with THC by other routes of administration. D. Holtzman, R. A. Lovell, J. H. Jaffe, D. X. Freedman, *Science* **163**, 1464 (1969); H. I. Bircher and R. Mechoulam, *Arch. Int. Pharmacodyn.* **172**, 24 (1968).
11. The procedure used was based on the availability of authentic reference standards for Δ^9THC and its corresponding 11-hydroxy and 8,11-dihydroxy metabolites. The R_F values for the authentic compounds were identical with those of the well-separated zones.
12. Incubation of Δ^9THC with human or rat livers results in the formation of 11-hydroxy-Δ^9THC and 8,11-dihydroxy-Δ^9THC in approximately the same proportions found in the case with mice.
13. Blood was taken by heart puncture from three mice at each time period. The administered dose was 0.2 mg of Δ^9[^{3}H]THC (25.2 μc/mg).
14. The labeled Δ^9- and Δ^8THC used were prepared at the Research Triangle Institute under contract PH-43-69-1452 with the NIMH. This study was supported by contract HSM-42-69-62 between the Center for Studies of Narcotic and Drug Abuse of the Division of Narcotic Addiction and Drug Abuse, NIMH, and the Research Triangle Institute.

17 November 1970

Microsomal Hydroxylase Induction in Liver Cell Culture by Phenobarbital, Polycyclic Hydrocarbons, and p,p′-DDT

Abstract. *Aryl hydrocarbon hydroxylase induction by phenobarbital, polycyclic hydrocarbons, and the insecticide, 2,2-bis(*p-*chlorophenyl)-1,1,1-trichloroethane* (p,p′-*DDT), occurs in rat fetal liver cell cultures. The maximum net rate at which the hydroxylase activity accumulates is about the same when phenobarbital, 3-methylcholanthrene, or benz*[a]*anthracene is in the growth medium at optimum concentrations. An additive effect is obtained when either phenobarbital or* p,p′-*DDT is present with a polycyclic hydrocarbon in the growth medium, but not when the cells are treated with phenobarbital plus* p,p′-*DDT or with the combination of two polycyclic hydrocarbons.*

The mixed-function oxygenases (*1*) are induced by at least two major classes of compounds (*2*). Compounds such as barbiturates, phenothiazines, and insecticides represent one class of inducers, and polycyclic hydrocarbons are examples of a second type. In vivo, phenobarbital (PB) stimulates the activity of many microsomal enzymes, and the maximum levels are reached after 3 days or more of repeated treatment; also, marked proliferative changes in the smooth endoplasmic reticulum are found (*2*). Polycyclic hydrocarbon administration as a single dose generally induces the activity of several specific oxygenases to peak levels in about 24 hours, and no marked proliferation of the microsomal membranes occurs (*2*). Using aryl hydrocarbon hydroxylase (*3*) as an enzyme inducible by either class of inducers, Nebert and Gelboin (*4*) found that hydroxylase induction by PB in vivo occurs to a significant extent only in liver. In contrast, hydroxylase induction by polycyclic hydrocarbons in vivo occurs rapidly in most mammalian tissues examined (*4*).

In fetal cell cultures derived from the entire hamster (*5–9*) or mouse (*10*), aryl hydrocarbon hydroxylase induction by polycyclic hydrocarbons dissolved in the growth medium takes place, whereas concentrations as high as 10 m*M* PB did not induce the enzyme (*7*). We have found (*11*), however, that treatment of cell cultures derived from the entire rat fetus with 2 m*M* PB elicits about a two-fold increase in hydroxylase activity. This finding is consistent with the possibility that in the heterogeneous cell population the enzyme system in one type of cells, such as hepatocytes, can be induced by PB. Thus, we now describe aryl hydrocarbon hydroxylase activity inducible in primary fetal liver cell cultures by various microsomal enzyme inducers. Such an experimental model in cell culture may be beneficial for comparing certain effects of drugs, insecticides, and polycyclic hydrocarbons on oxidase activity.

The cell culture materials and techniques were similar to those described (*6, 7*). Livers from rat fetuses about 14 to 18 days of age were used in the preparation of primary cultures of dispersed (*12*) cells. About 30 to 60 fetuses were used in a typical preparation of primary cultures; cells from male and female fetuses were pooled. Fetal liver tissue was dissected free from the surrounding tissues and minced with sterile scissors. After 10 minutes of treatment with Viokase (Grand Island) at room temperature (*8–10*), the mixture was filtered through sterile gauze. The hepatocytes and numerous red blood cells were then washed in Dulbecco's phosphate buffer, *p*H 7.1, and plated at a density of 4×10^6 to 8×10^6 cells per milliliter. The red cells were readily removed from the surface of the tissue culture dish 6 hours later, and at this time islands of hepatocytes had attached. Phenobarbital dissolved readily in the growth medium at concentrations as high as 5 m*M*. Benz[*a*]anthracene (BA), 3-methylcholanthrene (MC), and 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane (*p,p′*-DDT) were first dissolved in small amounts of dimethylsulfoxide, and this solution was then added to the growth medium. Control medium and PB-containing medium were treated