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BIOLOGICAL DISPOSITION OF TETRAHYDROCANNABINOLS[1,2]

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Despite many centuries of use and years of research, there has been a rather large "research gap" in our knowledge about marihuana (22). A primary area of ignorance has been the almost complete lack of data concerning the fate of its cannabinoids[3] or other components in body metabolism. This situation is unfortunate because adequate drug metabolism studies are a necessary foundation for comprehensive pharmacological and toxicological tests and for rational consideration of the fundamental questions of safety and legalization.

Before the confirmation in 1964 of (−)-*trans*-Δ^9-tetrahydrocannabinol (Δ^9-THC) as the primary active component in marihuana (11), only meager evidence was available concerning the biological fate of tetrahydrocannabinols. As early as 1946, Loewe (19) showed a transfer between dogs of an ataxia-producing blood extract. Miras (23) measured the distribution of radioactivity in organs of the rat after intraperitoneal injection of purified THC preparation from a plant grown in $^{14}CO_2$. He was the first to note two striking characteristics of THC, namely that it is poorly absorbed after intraperitoneal injection, and that it is highly concentrated in the liver. With the synthesis of Δ^8- and Δ^9-THC, and the labeling of these compounds first with ^3H (5, 24), and later with ^{14}C (27), metabolic studies became possible.

Before discussing the metabolic fate of marihuana, a preliminary consideration is needed of the fate of cannabinoids during pyrolysis into marihuana smoke since this is the principal way in which the drug is used in most countries. This review will endeavor to survey the information available at present concerning the changes during the smoking process as well as after entry into the body.

SMOKING STUDIES

The earliest reported studies on the composition of marihuana smoke apparently utilized plant material having an undocumented history and did not attempt to simulate the smoking patterns typical of a marihuana smoker (7–9, 25, 30). Smoking studies at Battelle have employed a smoking machine programmed to reproduce the puff interval, rate and volume characteristics taken from a large panel of experienced smokers (Foltz *et al.*, unpublished data). Generally these smoking studies have rectified certain early misconceptions and showed that the ratios of cannabinoids in smoke are similar to the ratios of

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[2] Presented in part at the First Midwestern Conference on Drug Metabolism University of Cincinnati, January, 1971.

[3] The term cannabinoid is intended to include the C_{21} compounds typical of and present in *Cannabis sativa* and their carboxylic acid precursors, analogues and biotransformation products.

cannabinoids found in the plant. The major exception to this is that the heat of combustion converts most, but not all, of the THC-acid precursors into THC. Contrary to early speculations, there is very little conversion of cannabidiol to THC by ring closure and only minimal isomerization of Δ^9-THC to Δ^8-THC as claimed by Lerner and Zeffert (18). The recovery of Δ^9-THC which has been added to reefers, made from exhaustively extracted plant material, has not substantiated the report by Claussen and Korte (8) that over 90% of the THC is lost through combustion. The average distribution of Δ^9-THC in smoke produced from the Battelle machine is shown in figure 1 (Foltz *et al.*, unpublished data). These data show that approximately 50% of the total THC dose is delivered, provided the butt is fully consumed, and these findings have been confirmed in another laboratory (20).

Absorption

Many investigators have encountered difficulty in detecting, much less quantifying, unchanged THC in body fluids by using non-radioactive techniques. This difficulty is chiefly due to the rapid conversion of THC to its metabolite (*vide infra*). THC has a half-life of only about 14 min in rabbits (2), 30 min in rats (16), and roughly 30 min in man based upon experiments with three normal subjects (17).

Smoke inhalation, the principal route of administration of marihuana in man, is rapid and efficient but difficult to quantify and to apply to animals. Marihuana is also widely consumed orally, and Isbell *et al.* (15) have shown that absorption from the gastrointestinal tract is only about one-third as effective as inhalation of smoke containing THC. Many pharmacological and toxicological

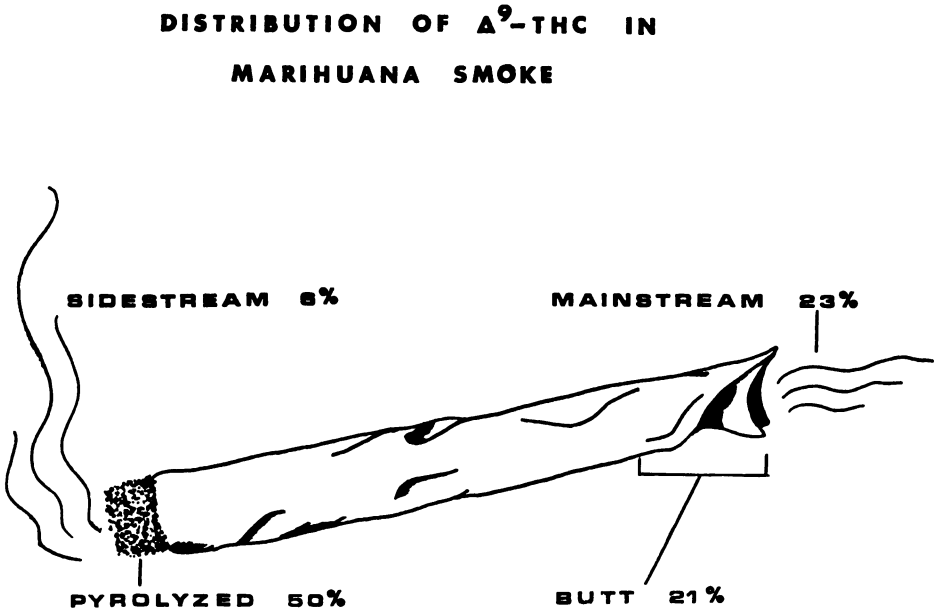


FIG. 1. Distribution of Δ^9-THC in marihuana smoke

studies point to slow and limited absorption of THC after intraperitoneal administration. The intravenous route, which is subject to limitations in chronic use and which is dissimilar to street use, remains the method of choice for short-term experiments.

The solubilization, micro-emulsification, or suspension of finely divided particles of THC is a necessary, but confounding, factor in the preparation of the drug for absorption. The agents used have included various surfactants (Tween 80, Triton X-100, Pluronic F-68), solvents (ethanol, propylene glycol, dimethylsulfoxide, glycerin, and peanut, olive, or sesame oils) and suspending agents (bovine serum albumin and gum arabic). Each of these agents may, in itself, influence the rate of absorption of THC and some may produce pharmacological effects. The wide variety of methods employed by different investigators makes quantitative comparisons difficult, and the use of oils or the suspension of unsolubilized THC microglobules appears to impede absorption.

Distribution

In addition to the early study by Miras, the distribution of radiolabeled compounds has been studied in animals (2, 12, 13, 16, 29), and in man (17). Most of these studies have used ^3H and report only the total radioactivity of tissues despite rapid metabolite formation (2, 4, 10, 32). Nevertheless, these data were useful in showing:

1. The rapid accumulation of radioactivity in the liver;
2. Biliary excretion and persistence of the drug and/or metabolites in the feces;
3. The ability of THC and/or metabolites to cross the placenta;
4. The portion of the drug and/or metabolites excreted *via* the kidney and urine;
5. The lack of preference of the drug and/or metabolites for brain tissue.

By inhalation, the distribution pattern was not remarkably different from the intraperitoneal route except for initial retention by the lung (12). In plasma, 80 to 95% of the Δ^9-THC migrates in association with lipoprotein (31).

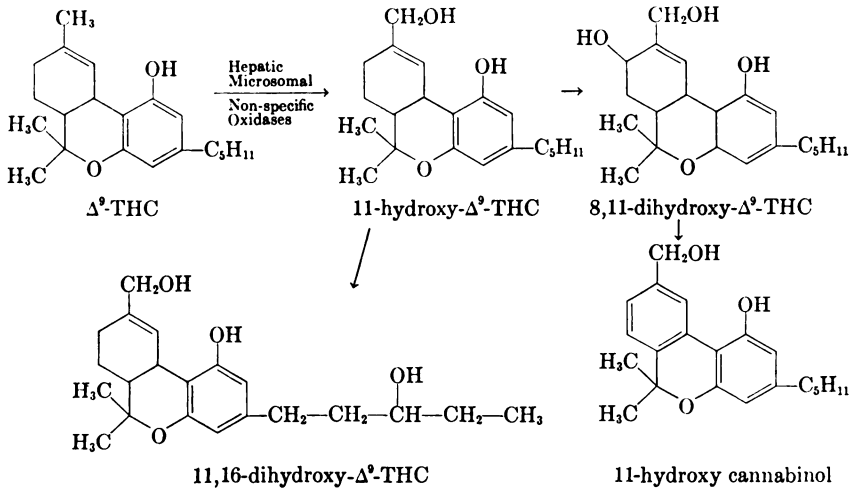
Metabolism

The hepatic accumulation noted above prompted investigators in this laboratory (10), and others (2, 4, 32), to add Δ^8- or Δ^9-THC to liver homogenates and then to the microsomal fraction to examine possible metabolism *in vitro*. Little metabolic alteration occurs in whole liver homogenates, but, when the high speed microsomal fraction (10,000 $\times$ *g*) is fortified with $NADPH_2$[4] regenerating system, a metabolite is rapidly formed which has been identified as 11-hydroxy-Δ^8-THC when Δ^8-THC was used as the precursor (4, 10), or 11-hydroxy-Δ^9-THC when Δ^9-THC was used as the precursor (24, 32). The metabolite of Δ^8-THC has also been isolated and identified in urine (4, 5).

The importance of these hydroxy-THC metabolites is emphasized by 1) their rapid formation (accounting for the difficulties of many investigators to detect

[4] $NADPH_2$, nicotinamide adenine dinucleotide phosphate-reduced form.

THC *in vivo*), 2) their pharmacological activities which strongly resemble Δ^8- and Δ^9-THC and exceed these precursors in potency, and 3) the parallel time courses of their presence and the duration of observable drug action. Wall and associates (32) have reported a further conversion of Δ^9-THC to 8,11-dihydroxy-Δ^9-THC and then to 11-hydroxy cannabinol. They have also detected sidechain oxidation at the *beta*-position. Some 11-hydroxy cannabinol is also formed from cannabinol (33). These biotransformations may be summarized in the following reactions scheme:



Undoubtedly, the biotransformation of THC is complex, and multiple products must be anticipated. However, the full significance of the metabolic events may well have other implications in addition to revealing the psychoactive metabolite. Tolerance to marihuana is suspected to decrease during initial exposures to the drug before it increases after chronic consumption of large amounts (21, 22). Thus, induction of hepatic microsomal enzymes may explain in part the increasing pharmacological effect of the drug during the first several exposures in addition to improved smoking technique and better recognition of the euphoric effect. Induction of microsomal non-specific oxidases is implied by the finding of a shortened barbiturate sleeping time in mice treated chronically with THC (28). Finally, a significant relationship may exist between persistence of dihydroxy-THC and hydroxy-cannabinol derivatives and the after-effects of THC on mood, motivation and thought processes.

Excretion

It is apparent that the apportionment of excretion products between the fecal and urinary routes will vary in different animal species (2). The presence of metabolites in the urine was detected before their identification (1, 6a). Only a very small portion of free-THC is excreted intact in the urine, but some is conjugated with glucuronic acid (2). At least three major metabolites appear in the urine of rabbits (2). These metabolites are more polar than THC and in the

case of Δ^8-THC one of these has been identified as 11-hydroxy-Δ^8-THC (4, 5). Conversion of the urinary 11-hydroxy-Δ^9-THC to cannabinol with *p*-toluenesulfonic acid may provide a method for detection of cannabis use (3).

The conversion of THC to more polar metabolites provides a method for differential extraction with diethyl ether (16). In rats, the major route of excretion for 11-hydroxy-Δ^9-THC is through the feces, whereas in rabbits urinary excretion predominates. In rats, the persistence of excretory products in the feces suggested an enterohepatic circulation which has been confirmed by Klausner and Dingell (16) who showed reabsorption of the metabolites after an intraduodenal injection of bile in an untreated recipient rat. Recycling of THC metabolites in this manner is reminiscent of the macrolide antibiotics and may also be related to the occurrence of gastrointestinal side effects by both groups of compounds.

Biological activity of metabolites

Fortuitously, a facile chemical conversion of Δ^8-THC to 11-hydroxy-Δ^8-THC has made available a supply of this metabolite in relatively pure form (>90% purity) (10). The author has examined the behavioral action of the synthetic metabolite in the rat by using a wide variety of test parameters (28) modified from Irwin's mouse behavioral screening procedures (14). The metabolite reproduces the complete pattern and time course of both Δ^8- and Δ^9-THC activities and is equal to or slightly more potent than these compounds by the intravenous route. Similar effects of all three drugs in the rat include cataleptic posturing (trance-like behavior), bizarre acts (retropulsion and backwards circling), abnormal biting, hyperstartle reactions, decreased spontaneous exploratory activity, vocalization in response to mild stimuli, and others. This same metabolite made by *de novo* synthesis also produces effects in the monkey which are similar to the parent drug (4). The metabolite of Δ^9-THC has not yet been achieved by synthesis, but relatively pure products isolated from hepatic microsomal preparations have shown THC-like behavioral activity in the mouse (24, 32). Indeed, after intracerebral injection, the potency of 11-hydroxy Δ^9-THC is about 18 times that of the precursor compound [Christensen *et al.* (6)].

On the basis of increased THC potentiation of barbital sleeping time by the microsomal metabolic inhibitor, SKF-525-A, Sofia and Barry (26) questioned the metabolite activity hypothesis at least for depressant effects of THC. However, preliminary study of this question by the author has indicated that this effect of SKF-525-A may be an influence of the drug on hepatic metabolism of barbital since the metabolite-barbital sleep time is also increased. It will be important to try the action of hydroxy-THC in man and to examine other major metabolites for possible contributions to the prolonged after effects of THC on mood, motivation and thought processes or other unsuspected toxic actions.

REFERENCES

1. Agurell, S., Nilsson, I. M., Ohlsson, A. and Sandberg, F.: Elimination of tritium-labelled cannabinols in the rat with special reference to the development of tests for the identification of cannabis users. Biochem. Pharmacol. 18: 1195–1201, 1969.
2. Agurell, S., Nilsson, I. M., Ohlsson, A. and Sandberg, F.: On the metabolism of tritium-labelled Δ^1-tetrahydrocannabinol in the rabbit. Biochem. Pharmacol. 19: 1333–1339, 1970.

3. Andersen, J. M., Nielsen, E., Schou, J., Steentoft, A. and Worm, K.: A specific method for demonstration of cannabis intake by TLC of urine. Acta Pharmacol. Toxicol. **29**: 111–112, 1971.
4. Ben-Zvi, Z., Mechoulam, R. and Burstein, S.: Identification through synthesis of an active $\Delta^{1(6)}$-tetrahydrocannabinol metabolite. J. Amer. Chem. Soc. **92**: 3468–3469, 1970.
5. Burstein, S. and Mechoulam, R.: Stereospecifically labeled $\Delta^{1(6)}$-tetrahydrocannabinol. J. Amer. Chem. Soc. **90**: 2420–2421, 1968.
6. Christensen, H. D., Freudenthal, R. D., Gidley, J. D., Rosenfeld, R., Boegli, G., Testino, L., Brine, D. R., Pitt, G. G. and Wall, M. E.: Activity of Δ^8 and Δ^9 tetrahydrocannabinol and related compounds in the mouse. Science **172**: 165–167, 1971.
6a. Christensen, J. and Rafaelsen, O. J.: Cannabis metabolites in urine after oral administration. Psychopharmacologia **15**: 60–63, 1969.
7. Claussen, U. and Korte, F.: Hashish. XIII. Behavior of constituents of *Cannabis sativa* during smoking (in German). Tetrahedron Lett. No. 22: 207, 1967.
8. Claussen, U. and Korte, F.: Hashish: Behavior of hemp and Δ^9-6a-10a-transtetrahydrocannabinol during smoking. Ann. Chem. (Justus Liebigs) **713**: 162–165, 1968.
9. Coutselinis, A. and Miras, C. J.: U. N. Secretariat Document ST/SOA/SER S/23, October 30, 1970.
10. Foltz, R. L., Fentiman, A. F., Jr., Leighty, E. G., Walter, J. L., Drewes, H. R., Schwartz, W. E., Page, T. F., Jr. and Truitt, E. B., Jr.: Metabolite of (−)-trans-Δ^8-tetrahydrocannabinol: identification and synthesis. Science **168**: 844–845, 1970.
11. Gaoni, Y. and Mechoulam, R.: Isolation, structure and partial synthesis of active constituent of hashish. J. Amer. Chem. Soc. **86**: 1646–1647, 1964.
12. Ho, B. T., Fritchie, G. E., Kralik, P. M., Englert, L. F., McIsaac, W. M. and Idänpään-Heikkila, J.: Distribution of tritiated-*l*-Δ^9-tetrahydrocannabinol in rat tissue after inhalation. J. Pharm. Pharmacol. **22**: 538–539, 1970.
13. Idänpään-Heikkila, J., Fritchie, G. E., Englert, L. F., Ho, B. T. and McIsaac, W. M.: Placental transfer of tritiated-*l*-delta-9-tetrahydrocannabinol. N. Engl. J. Med. **281**: 330, 1969.
14. Irwin, S.: Comprehensive observational assessment: Ia. A systematic, quantitative procedure for assessing the behavioral and physiological state of the mouse. Psychopharmacologia **13**: 222–257, 1968.
15. Isbell, H., Gorodetzsky, C. W., Jasinski, D., Claussen, U., Spulak, F. V. and Korte, F.: Effects of (−)Δ^9-transtetrahydrocannabinol in man. Psychopharmacologia **11**: 184–188, 1967.
16. Klausner, H. A. and Dingell, J. V.: The metabolism and excretion of Δ^9-tetrahydrocannabinol in the rat. Life Sci. **10** (Pt. 1): 49–59, 1971.
17. Lemberger, L., Silberstein, S. D., Axelrod, J. and Kopin, I. J.: Marihuana: studies on the disposition and metabolism of delta-9-tetrahydrocannabinol in man. Science **170**: 1320–1322, 1970.
18. Lerner, M. and Zeffert, J. T.: Determination of tetrahydrocannabinol isomers in marihuana and hashish. Bull. Narc. **20**: 53–54, 1968.
19. Loewe, S.: Studies on the pharmacology and acute toxicity of compounds with marihuana activity. J. Pharmacol. Exp. Ther. **88**: 154–161, 1946.
20. Manno, J. E., Kiplinger, G. F., Bennett, I. F., Haine, S. and Forney, R. B.: Comparative effects of smoking marihuana or placebo on human motor and mental performance. Clin. Pharmacol. Ther. **11**: 808–815, 1970.
21. McMillan, D. E., Harris, L. S., Frankenheim, J. M. and Kennedy, J. S.: *l*-Δ^9-trans-tetrahydrocannabinol in pigeons: tolerance to the behavioral effects. Science **169**: 501–503, 1970.
22. Mechoulam, R.: Marihuana chemistry. Science **168**: 1159–1166, 1970.
23. Miras, C. J.: Some aspects of cannabis action. *In* Hashish: Its Chemistry and Pharmacology, Ciba Foundation Study Group No. 21, ed. by G. E. W. Wolstenholme and J. Knight, pp. 37–58, Little, Brown & Co., Boston, 1965.
24. Nilsson, J. L. G., Nilsson, I. M. and Agurell, S.: Synthesis of ^{3}H- and ^{14}C-labeled tetrahydrocannabinols. Acta Chem. Scand. **23**: 2209–2211, 1969.
25. Shoyama, Y., Yamaguchi, A., Sato, T., Yamaguchi, T. and Nishioka, I.: Cannabis. IV. Smoking test (in Japanese; English title from Chem. Abstr. **71**: 89975h, 1969). Yakugaku Zasshi **89**: 842–845, 1969.
26. Sofia, R. D. and Barry, H., III: Depressant effect of Δ-*l*-tetrahydrocannabinol enhanced by inhibition of its metabolism. Eur. J. Pharmacol. **13**: 134–137, 1970.
27. Timmons, M. L., Pitt, C. G. and Wall, M. E.: Deuteration and tritiation of Δ^8- and Δ^9-tetrahydrocannabinol. The use of trifluoroacetic acid as a convenient labeling reagent. Tetrahedron Lett. No. 36: 321–932, 1969.
28. Truitt, E. B., Jr.: Pharmacological activity in a metabolite of *l*-trans-Δ^8-tetrahydrocannabinol (abstr.). Fed. Proc. **29**: 619, 1970.
29. Turk, R. F., Manno, J. E., Jain, N. C. and Forney, R. B.: LD_{50} and distribution of pure (99+%) natural tetrahydrocannabinol in rats (abstr.). Pharmacologist **11**: 280, 1969.
30. Vieira, F. J., Aguiar, M. B., Alencar, J. W., Seabra, A. P., Tursch, B. M. and Leclercq, J.: Effects of the organic layer of hashish smoke extract and preliminary results of its chemical analysis. Psychopharmacologia **10**: 361–362, 1967.
31. Wahlqvist, M., Nilsson, I. M., Sandberg, F., Agurell, S. and Grandstand, B.: Binding of Δ^1-tetrahydrocannabinol to human plasma proteins. Biochem. Pharmacol. **19**: 2579–2584, 1970.
32. Wall, M. E., Brine, D. R., Brine, G. R., Pitt, C. G., Freudenthal, R. I. and Christensen, H. D.: Isolation, structure, and biological activity of several metabolites of Δ^9-tetrahydrocannabinol. J. Amer. Chem. Soc. **92**: 3466–3468, 1970.
33. Widman, M., Nilsson, I. M., Nilsson, J. L. G., Agurell, S. and Leander, K.: Metabolism of cannabis. IX. Cannabinol: structure of a major metabolite formed in rat liver. Life Sci. **10** (Pt. II) **10:1** 157–162, 1971.