Analysis of Cannabinoids
Research Monograph 42, R. Hawks, Editor
National Institute on Drug Abuse, 1982

The Constituents of Cannabis and the Disposition and Metabolism of Cannabinoids

Richard L. Hawks

INTRODUCTION

Humans consume cannabinoids primarily by smoking preparations of Cannabis sativa L. When cannabis plant material is prepared for purposes of intoxication, it is referred to as "marijuana." Cannabis that is used as an intoxicant comes from many different geographical sources around the world and is referred to as "drug type" cannabis because it contains significant quantities of Δ^9-tetrahydrocannabinol (THC), the primary chemical ingredient of the plant material responsible for the psychopharmacological effect. Another type of cannabis equally prevalent worldwide is referred to as "hemp type" because originally, about four centuries ago, it was cultivated for the manufacture of rope material. Cannabis is not in fact a plant indigenous to the New World; it was introduced to the Western Hemisphere by Spanish explorers, who used large amounts of hemp rope in their shipping industry.

More than 60 cannabinoid compounds have been identified in the "drug type" cannabis used illicitly today. Some of the more important ones are illustrated in figure 1. In addition, as in any plant material, there is a large number of other chemical compounds present, several hundred of which have been identified in cannabis (1).

Though cannabis contains many cannabinoid components and other chemical substances, THC is the most prominent psychoactive cannabinoid component, and its concentration determines the "potency" of marijuana. Other cannabinoids may contribute to THC's activity through interactions or direct effects on certain specific pharmacologic measures, but THC is the compound on which

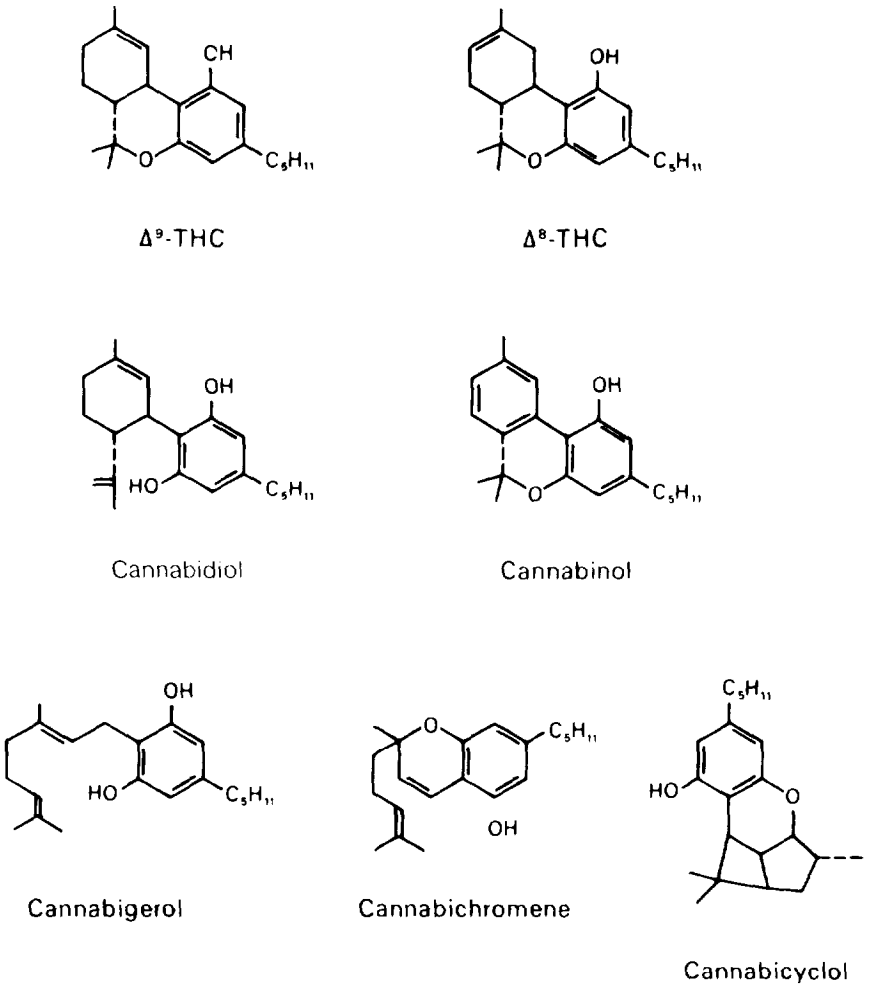


FIGURE 1. Cannabinoids of primary interest found in Cannabis Sativa.

most studies to date have concentrated. The psychopharmacologic activities of either cannabinol or cannabidiol, for instance, are estimated to be less than 10% as great in man as that of THC itself (2). The predominant factor affecting potency is the genetic stock of the plant. Seeds derived from cannabis of relatively high THC content will produce plants of relatively high potency, other factors being equal. It should also be noted that the relative amounts of cannabinoids in the plant can vary significantly depending on how and where the plant is grown and harvested. Soil, climate, cultivation, and age can have considerable effect on the final "product."

Samples of marijuana from illegal shipments, confiscated between 1979 and 1982 by the Drug Enforcement Administration and analyzed by the NIDA potency monitoring program, averaged above 3.0% THC by weight. Potency has increased significantly since the program started in 1974, when confiscated material was found to contain less than 0.5%. A cannabis preparation called "sinsemilla" has become increasingly available over the last 4 years and is currently averaging about 6% THC. Occasional samples with potencies as high as 10% have been encountered. The potential for ingesting psychologically dysphoric amounts of THC is thus much increased with the availability of these more potent forms of cannabis.

The THC in marijuana is predominantly in the form of Δ^9-THC-2-(and a small amount of 4-)-carboxylic acid (also called "tetrahydrocannabinolic acids" or "THC-acids") (1). These THC-acids are changed rapidly into THC itself when they are subjected to the heat of the burning cigarette. The burning process also causes pyrolytic destruction of a portion of the THC and THC acid in the cigarette. The actual amount of THC delivered in the smoke has been estimated at 20% to 70% in experiments conducted with smoking machines (3). The estimate of 70% is derived from a study in which the cigarette was burned with a continuous draw, and 20% was found when a 5-second puff each minute was used. The latter rate is the standard for tobacco smoking experiments. Marijuana smokers probably fall in between these estimates since they usually inhale longer and more frequently than once a minute for 5 seconds. This assumption is supported by a study which determined that the bioavailability of THC from marijuana cigarettes as measured in blood of human subjects ranged from 8% to 24% (mean 18±6%) in 11 subjects (4). This "bioavailability" is the fraction of THC in the cigarette which reaches the bloodstream. If for instance, 35% of the THC in the cigarette were delivered in the smoke and 50% of this passed through the lung to the blood, a bioavailability of 17.5% would result, which is within the reported range.

For the past 10 years NIDA has supplied cannabis cigarettes for use in animal and human research on marijuana toxicity, pharmacology, pharmacokinetics, and chemistry. The cigarettes are sup plied in potencies ranging from 0.5% to 3% by weight. The plant material is grown under controlled conditions, and the material is chemically characterized by quantitative analysis of eight major cannabinoids. The cigarettes are supplied in a form similar in size to unfiltered tobacco cigarettes.

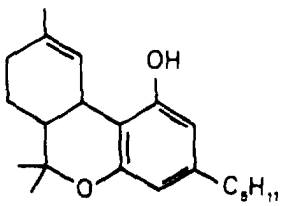
Other THC dosage forms developed by NIDA and made available for research include oral doses of THC in sesame oil contained in gelatine capsules, intravenous solutions of THC which are either suspended in human serum albumen or dissolved in ethanol, and ophthalmic preparations of THC in mineral oil. Each of these dosage forms has its own unique time course of effect and metabolic profile. Even though these dosage forms were developed for use in research directed at consequences of marijuana abuse, they have also served as dosage forms for studies in several areas of therapeutic research. The oral form is under investigation as an antiemetic drug for patients undergoing cancer chemotherapy, and the ophthalmic preparation is being investigated as a treatment to relieve intraocular pressure resulting from glaucoma.

METABOLISM AND DISPOSITION

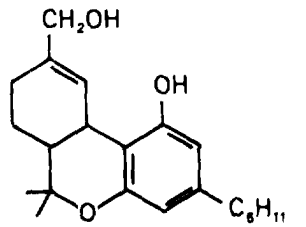
When cannabis is smoked or ingested orally, THC and the many other cannabinoids present undergo extensive metabolism into many oxygenated species. Most of the research on cannabinoid metabolism has been carried out on THC itself. Metabolites of THC which have been characterized and isolated from man (5,6,7) are illustrated in figure 2.

The total number of metabolites resulting from human administration of THC is undoubtedly much greater. In vitro and in vivo animal studies indicate that a large number of hydroxylated and carboxylated metabolites result from the metabolism of THC as well as from the other cannabinoids found in the plant (8-14). Investigators have also shown the presence of fatty acid conjugates of THC (15,16), a variety of 0-glucuronides (17,18), and C-glucuronides (19).

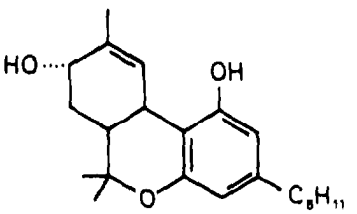
The formation of glucuronide conjugates of THC and its metabolites has been reported to occur on the phenolic position (17), on the carboxylic acid position in 11-nor-Δ^9-THC-9-carboxylic acid (9-carboxy-THC) (18), and directly on the two and four positions of the aromatic ring (19). It is unclear at this time how this conjugation affects excretion, storage, and recirculation of material, but it is clear that a major portion of the cannabinoid metabolites excreted in urine are in the form of glucuronide conjugates (5,18,20). In the case of 9-carboxy-THC, conjugation may occur at both the phenolic and the carboxy position, thus leading to the possibility of three different conjugated species. A recent study has provided the characterization of 12 additional acid metabolites of THC (21). An extensive and complex cannabinoid metabolic profile in man is indi-



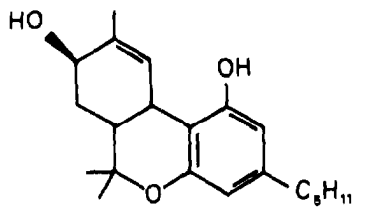
Δ⁹ THC
(active)



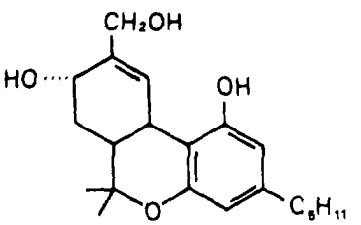
11-Hydroxy-Δ⁹ THC
(active)



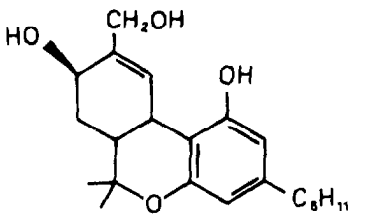
8α-Hydroxy-Δ⁹ THC
(much less active)



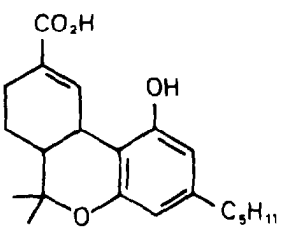
8β-Hydroxy-Δ⁹ THC
(less active)



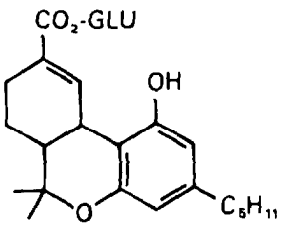
8α, 11-Dihydroxy-Δ⁹ THC



8β, 11-Dihydroxy-Δ⁹ THC



11-nor-Δ⁹ THC-9-
Carboxylic Acid
(inactive)



11-nor-Δ⁹ THC-9-Carboxylic
Acid Glucuronide

cated when all the possible cannabinoid metabolites and their respective conjugates are considered.

The metabolite concentrations in figure 3 are typical of the significant differences found in blood after oral administration (22) of THC as compared with administration via smoking (23). The 11-hydroxy metabolite probably plays a minor role after smoking as the concentration is very low (5), less than 10% of the THC concentration at any time. After oral administration, however, 11-OH-THC may play a more important role as its concentration is relatively higher in blood, often higher than that of THC. Significant amounts of THC are not observed after oral administration owing primarily to the relatively slow absorption of THC and subsequent extensive metabolism via the "first pass" through the liver. The blood profile of the oral dose indicates 9-carboxy-THC as the predominant metabolite.

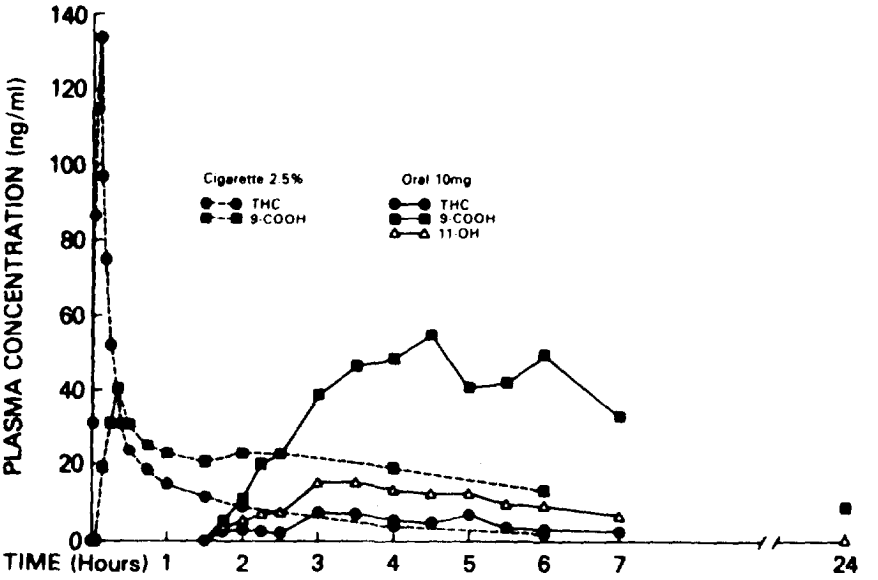


FIGURE 3. Plasma concentration versus time curve for THC and its metabolites after oral and smoked administrations.

Several of the hydroxylated metabolites have been shown to have some activity (24), but 9-carboxy-THC has shown little or no activity when administrated to either man (24) or animal. The only active metabolite known to occur in any significant concentration is 11-hydroxy-Δ^{9}-THC, which has been shown to have activity equal to or perhaps slightly greater than THC itself when administered alone to man (25). The psychopharmacological activity in man of 8-

beta-hydroxy-THC has been reported as about 4% of that of Δ^9-THC and 8-alpha-hydroxy-THC less than 10% (24).

In human studies it has been shown that 80% to 90% of the total dose of THC administered is excreted within 5 days, mostly (65%) in feces (26). The urinary fraction, which makes up the remaining 18% to 23% of the dose excreted, consists primarily of acidic metabolites such as 9-carboxy-THC and other related acids (21). Only about 5% of the total urinary fraction consists of neutral cannabinoids, and THC itself makes up less than 1 % of this. The endpoint of the renal pathway is therefore multi-oxygenated acidic metabolites. The feces fraction consists of approximately equal parts neutral and acidic metabolites, 9-carboxy-THC making up about 29% of the extracted metabolites, THC about 8%, and 11-hydroxy-THC about 21% (5).

When the time course of the disappearance of THC from plasma is compared with the very slow excretion of metabolites in urine, it becomes obvious that some mechanism is contributing to this retarded elimination of material. As mentioned above, 80% to 90% of the THC dose administered IV is accounted for in 5 days, the remainder being eliminated slowly over a longer period of time. One mechanism which probably contributes significantly to the long excretion of these metabolites involves enterohepatic recirculation, since the major portion of IV doses in humans is eliminated in the feces (26) and reabsorption of biliary excreta has been demonstrated in the dog (27). Other mechanisms which could contribute include renal reabsorption and deeptissue distribution. Because of the lipid solubility (28) of THC, a characteristic shared by many of its metabolites, some portion of the dose is probably trapped in fatty-tissue pools for slow release. Preferential absorption of THC into the fat tissue of rats has been reported, while other tissues such as brain, lung, and muscle absorbed a much smaller proportion of the dose (29).

It is important to define both the mechanisms involved in this slow excretion and what specific metabolites are involved. Even though the acid metabolites are not likely to have psychoactivity, other types of toxic effects cannot be excluded. The characterization of fatty-acid conjugates of THC has led to speculation that species of this type may contribute to storage of cannabinoids in body tissue (15,161. Much research remains to be done to ascertain the chronic toxicity of these or the many other THC metabolites.

The character of the metabolites and their mode of elimination is important since the elimination process is measured in days, not hours, and repeated administration via marijuana smoking likely

leads to accumulation of both THC and metabolites and the possibility of subsequent toxic consequences.

Much of the forensic interest in cannabinoid analysis of body fluids has focused on the 9-carboxy-THC metabolite, the principal acid metabolite of THC that has been characterized and synthesized for study. This metabolite is an important species in both blood and urine. As illustrated in figure 3, THC levels which are initially quite high drop very rapidly and cross the rising 9-carboxy-THC curve at approximately 20 minutes. Although this curve is based on a single dose, it does give credence to the hypothesis that a blood analysis which shows similar concentrations for both THC and 9-carboxy-THC could be an indication of very recent administration of marijuana and perhaps of a high probability that the subject is in an intoxicated state. This figure also illustrates that, because of the rapid decrease in the concentration of THC in blood, analytical methodology less sensitive than 1 or 2 nanograms per milliliter is not likely to detect the presence of THC after several hours except perhaps in a heavy chronic smoker. The curve for 9-carboxy-THC in this figure shows a considerably longer time course and therefore provides a better indicator of previous smoking.

Urine metabolite assays which have recently become available on the commercial market have made cannabinoid detection available to a much broader spectrum of laboratories including those associated with the armed forces, with private industry, and penal institutions. These methods include the SYVA Co. EMIT system and the Roche Diagnostics ABUSCREEN RIA system. The analysis of urine using these types of assays provides an indication of past use of marijuana. Due to the relatively long detection period of cannabinoid metabolites in urine, this ‘past use’ cannot be more specifically pinpointed than between 1 hour and 1 week. This makes any urine analysis, regardless of the levels detected, inadequate for prediction of intoxication or specific time of use.

As mentioned above, a blood analysis does have the potential for providing information relevant to intoxication. Blood samples are, however, difficult to obtain for many forensic purposes because of their invasive nature. Saliva and breath are much easier samples to acquire for drug detection and both have been investigated for the presence of marijuana components after smoking. The use of TIC methods for detection of THC in saliva has been suggested by several authors (30-34).

A study in humans using radiolabeled THC administered by intravenous injection (35) failed to detect any radioactivity in saliva samples. This suggests that neither THC or its metabolites pass

into the saliva or lung from the blood and that cannabinoids de tected in these biological matrices are sequestered during the smoking process.

In a recent study, an RIA specific for THC was used (36) to analyze a set of saliva samples (37) from subjects who smoked marijuana cigarettes containing cannabis of approximately 2% potency. Mixed saliva samples were taken from these subjects over a 24-hour period. The concentration of sequestered THC in these samples, illustrated in figure 4, is subject to considerably more fluctuation over time than plasma levels due to the inherent variability of mixed saliva concentration and to the difficulty in precisely aliquoting from a mixed saliva sample. It is clear, however, that significant levels of THC remain in saliva for several hours after smoking marijuana. During the first hour, in the absence of food intake, some levels exceeded 100 ng/ml and levels above 5 ng/ml

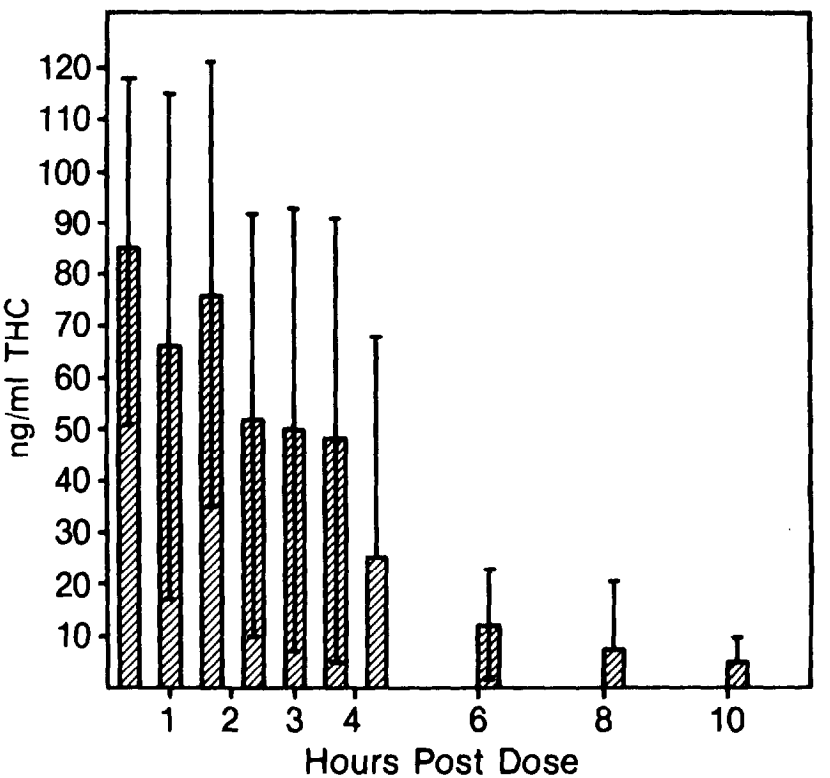


FIGURE 4. Human saliva levels after smoking a single marijuana cigarette containing 18 mg THC. N=8 at 20 min., N=7 at 60 min., N=5 for others. Variability expressed as standard deviation.

were still detectable in most samples beyond 8 hours even though normal food and liquid intake was resumed after 3 hours.

These results give further indication that saliva could be a useful fluid for presumptive detection of recent use. Even though no quantitative correlation can be made between saliva levels and intoxication since only sequested THC is being detected, the capacity to reduce the "past use" time frame from weeks to hours could have useful research and forensic applications.

The use of breath as a screening medium has been investigated using solvent and cryogenic traps with subsequent analysis by HPLC/MS (38) and also using solid matrix traps followed by conventional RIA (39). The levels in breath appear to be lower than those found in saliva after smoking, and use of such a medium for detection would consequently require relatively more sensitivity in the assay method as well as a more complex means of sample collection.

PHARMACOKINETICS

Studies of the pharmacokinetics of THC have been receiving increasing emphasis in the last few years as analytical techniques for the quantitative determination of cannabinoids in biological fluids have improved. The pharmacokinetics of THC and its metabolites is important to several research areas. A variety of studies have suggested that long-term, chronic use of marijuana may lead to adverse health effects in the individual (40). Some of these effects could be related to a toxic accumulation of either THC or metabolites of THC in the body. Pharmacokinetic studies of the mechanisms by which these cannabinoids are eliminated from the body are important in understanding the potential extent and rate of this accumulation.

Detailed pharmacokinetic studies of THC in humans under both acute and chronic dosing schedules are beginning to provide the basic data that will allow blood levels of active drug to be related to psychotomimetic, behavioral, and physiological effects (4,23,26). With the increasing availability of analytical methods for analysis of cannabinoids in blood and urine, there is increasing interest in providing analytical data for forensic purposes which can be related to the intoxication of individuals involved in accidents, as well as individuals who are legally liable for drug-taking behavior in the armed forces, on probation, or in industry. It is important to understand the kinetic mechanisms involved with THC and other cannabinoids in order to relate these forensic questions to the

meaning of a given blood level, particularly in cases where intoxication is a factor. The availability of more sensitive and more accessible analytical technology for the quantitative analysis of marijuana components in body fluids will play a key role in these efforts.

REFERENCES

1. C.T. Turner, M.A. Elsohly, and E.G. Boeren. Constituents of Cannabis Sativa L. XVII. A review of the natural constituents. *J. Natural Products, 43,* 169-234 (1980).
2. M. Perez-Reyes, M.C. Timmons, K.H. Davis, and M.E. Wall. A comparison of the pharmacological activity in man of intravenously administered delta-9-THC, cannabinol and cannabidiol. *Experientia, 29,* 1368-1369 (1973).
3. K.H. Davis, Research Triangle Institute, private communication.
4. A. Ohlsson, J-E. Lindgren, A. Wahlen, S. Agurell, L.E. Hollister, and H.K. Gillispie. Plasma delta-9-THC concentrations and clinical effects after oral and intravenous administration and smoking. *Clin. Pharm Ther., 98,* 409-1413 (1980).
5. M.E. Wall, D.R. Brine, and M. Perez-Reyes. Metabolism of cannabinoids in man. In *The Pharmacology of Marijuana, Vol. 1,* M.C. Braude and S. Szara, eds., Raven Press, New York, 93-116 (1976).
6. M.E. Wall, D.R. Brine, C.G. Pitt, and M. Perez-Reyes. Identification of delta-9 THC and metabolites in man. *J. Am. Chem. Soc., 94,* 8579-8681 (1972).
7. L. Lemberger, S.D. Silberstein, J. Axelrod, and I.J. Kopin. Delta-9-THC in man. *Science, 170,* 1320-1322 (1970).
8. S.H. Burstein, F. Menezes, E. Williamson, and R. Mechoulam. Metabolism of delta-l(6)-THC, an active marihuana constituent. *Nature, 225,* 87-88 (1979).
9. I.M. Nilsson, S. Agurell, J.L.G. Nilsson, A. Ohlsson, F. Sandberg, and *M.* Wahlqvist. Delta-1-THC: structure of a major metabolite. *Science, 168,* 1228-1229 (1970).
10. M.E. Wall, D.R. Brine, G.A. Brine, C.G. Pitt, R.I. Freudenthal, and H.D. Christensen. Isolation, structure, and biological activity of several metabolites of delta-9-THC. *J. Am. Chem. Soc., 92,* 3466-3468 (1970).
11. S. Agurell, I.M. Nilsson, M. Widman. Cannabinoids metabolites hydroxylated in the pentyl side chain. In *Marihuana Chemistry, Biochemistry and Cellular Effects,* G. Nahas, ed., Springer-Verlag, New York, 141-158 (1976).
12. S. Burstein. A survey of the metabolic transformations of delta-1-THC. In *Cannabinoid Analysis in Physiological Fluids.* J.A. Vinson, ed., American Chemical Society, Washington, D.C., 1-12 (1979).
13. M.E. Wall and D.R. Brine. Applications of mass spectrometry in cannabinoid research. In *Marihuana: Biological Effects,* G. Nahas and W.D.M. Paton, eds., Pergamon Press, London, 15-43 (1979).
14. D.J. Harvey, B.R. Martin, and W.D.M. Paton. Identification and measurement of cannabinoids and their in vivo metabolites in liver by gas chromatography-mass spectrometry. In *Marihuana: Biological Effects,* G.G. Nahas and W.D.M. Paton, eds., Pergamon Press, London, 45-62 (1979).
15. E.G. Leighty, A.F. Fentiman, and R.E. Foltz. Long retained metabolites of delta-9-THC identified as novel fatty acid conjugates. *Res. Commun Chem. Pathol. Pharmacol., 14,* 13-23 (1976).

16. W. Yisak, S. Agurell, J-E. Lingren, and M. Widman. In vivo metabolites of cannabinol identified as fatty acid conjugates. *J. Pharm. Pharmacol., 30, 462-463* (1978).
17. S. Pallante, M.A. Lyle, and C. Fenselau. Synthesis and characterization of glucuronides of 5'-hydroxy-delta-9-THC and 11-hydroxy-delta-9-THC. *Drug Metab. Dispos., 6,* 389-395 (1978).
18. P.L. Williams and A.C. Moffat. Identification in human urine of delta-9-THC-11-oic acid glucuronide: A THC metabolite. *J Pharm. Pharmacol., 32,* 445-448 (1980).
19. S. Levy, B. Yagen, and R. Mechoulam. Identification of a C-glucuronide of delta-6-tetrahydrocannabinol as a mouse liver conjugate in vivo. *Science, 200,* 1391-1392 (1978).
20. S.L. Kanter, L.E. Hollister, and J.U. Zamora. Marijuana metabolites in urine of man XI. Detection of unconjugatad and conjugated delta-9-THC-11-oic acid by thin layer chromatography. *J. Chromatogr., 235,* 507-512 (1982).
21. M.M. Halldin, S. Carlsson, S.L. Kanter, M. Widman, and S. Agurell. Urinary metabolites of Δ^1-tetrahydrocannabinol in man. Arzneimittelforschung, in press.
22. H. Gross, M.H. Ebert, V.B. Faden, S.C. Goldberg, W.H. Kaye, E.D. Caine, R.L. Hawks, and N. Zinberg, A double-blind trial of delta-9-tetrahydrocannabinol in primary anorexia nervosa. Submitted for publication, 1982.
23. M. Perez-Reyes, S. DiGuiseppi, K.H. Davis, V.H. Schindler, and C.E. Cook. Comparison of effects of marihuana cigarettes of three different potencies. *Clin. Pharmacd Ther., 31,* 617-624 (1982).
24. M. Perez-Reyes, M.D. Timmons, M.A. Lipton, H.D. Christensen, and K.H. Davis. A comparison of the pharmacological activity of delta-9-THC and its monohy droxylated metabolites in man. *Experientia, 29,* 1009-1010 (1973).
26. M. Perez-Reyes, M.C. Timmons, M.A. Lipton, K.H. Davis, and M.E. Wall. Intravenous injection in man of delta-9-THC and 11-OH-delta-9-THC, *Science, 177;* 633-635 (1972).
26. A.C. Hunt and R.T. Jones. Tolerance and disposition of THC in man. *J. Pharm. Exp.. Ther., 215,* 35-44 (1980).
27. E.R. Garrett and C.A. Hunt. Pharmacokinetics of delta-9-THC in dogs. *J. Pharm. Sci., 66,* 395-407 (1977).
28. E.R. Garrett and C.A. Hunt. Physicochemical properties, solubility, and protein binding of delta-9-THC. *J. Pharm. Sci, 63,* 1056-1064 (1974).
29. D.S. Kreuz and J. Axelrod. Delta-9-tetrahydrocannabinol: localization in body fat. *Science, 179,* 391-393 (1973).
30 I.S. Forrest, D.E. Green, S.E. Rose, G.C. Skinner, and D.M. Torres. Flourescent-labeled cannabinoids. *Res. Commun. Chem. Pathol. Pharmacol, 2,* 787-792 (1971).
31. A.P. Melikian and I.S. Forrest. Dansyl derivatives of delta-9- and delta-& tetrahydrocannabinols. *J. Pharm Sci., 62,* 1025-1026 (1973).
32. W.W. Just, G. Werner, and M. Weichmann. Bestimmung von delta-l- and delta-1(6) Tetrahydrocannabinol in Blut, Urin und Speichel von Haschisch-Rauchern. *Naturwiss., 59,* 222-223 (1972).
33. F. Friedrich-Fiechtl, G. Spiteller, W.W. Just, G. Werner, and M. Weichmann. Au Nachweis und Identifizierung von Tetrahydrocannabinol in Biologischen Flussighkeiten. *Naturwiss., 60,* 207-208 (1973).
34. W.W. Jut, N. Filipovic, and G. Werner. Detection of delta-9-THC in saliva of men by means of thin-layer chromatography and mass-spectrometry. *J. Chromatogr., 96,* 189-194 (1974).
36. M. Perez-Reyes, University of North Carolina, personal communication.

36. C.E. Cook, Research Triangle Institute, NC, performed under NIDA contract 271-80-3705.
37. H. Moskowitz, Southern California Research Institite, CA, samples collected under NIDA contract 271-76-3316.
38. J.L. Valentine, P.J. Bryant, P.L. Gutshall, O.H.M. Gan, and H.C. Niu. Detection of delta-9-THC in human breath following marijuana smoking. *Anal. Letters, 12* (B8) 867-880 (1979).
39. J.R. Soares, J.D. Grant, and S.J. Gross. Significant developments in radioimmune methods applied to delta-9-THC and its 9-substituted metabolites This volume.
40. *Marijuana and Health.* Report of a study by a Committee of the Institute of Medicine, AS. Relman, chairman. National Academy Press, Washington, D.C., 1982.

AUTHOR

Richard L. Hawks
Research Technology Branch
Division of Research
National Institute on Drug Abuse
Rockville, Maryland 20857